Ecophysiology of habitat-forming seaweeds in a changing environment

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Declarations

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Abstract

'Ecosystem engineering' species play a disproportionately important role in ecological systems by modifying (creating or destroying) habitat for other species. Understanding the impact of climate change is particularly critical for habitat-forming ecosystem engineers, as these species are generally facilitative and form the basis of hierarchically organised communities. In temperate marine environments, seaweed beds provide the fundamental structure of most shallow rocky reef communities. The coastline of southeastern Australia is warming at nearly four times the global average rate, with temperature increases occurring simultaneously with reduced nutrient availability due to strengthening seasonal incursions of warm, oligotrophic East Australian Current water. Climate change-driven range contractions in this area have already been documented for the canopy-forming *Ecklonia radiata* and *Phyllospora comosa*, while the areal coverage of *Macrocystis pyrifera* forests in Tasmania has declined by ~95% over the last 3-4 decades. Continued climatic change in this region is likely to impact on the physiological performance, reproductive capacity, survival, and ultimately the distribution of important habitat-forming seaweeds.

Predicting the future impacts of climate change on species distributions has largely focused around the use of bioclimate envelope models (BEMs), which relate field observations of a species' geographic distribution with environmental predictor variables to forecast its future distribution. This generalised modelling approach relies on the assumption that the physiological performance of a species under a given set of environmental conditions will be consistent across its range. However, Elser's Growth Rate Hypothesis (GRH) predicts that the performance of photosynthetic organisms will vary with latitude due to increased selective pressure for rapid growth at high latitudes resulting from a growth season that is shortened by low light availability. Determining whether the GRH is applicable to habitat-forming seaweeds is a critical step to evaluating the efficacy of BEMs in predicting the future impacts of climate change on seaweed distributions.

This thesis assesses the likely impacts of climate change on the physiology of the key biogenic habitat-forming seaweeds *E. radiata, P. comosa* and *M. pyrifera* in southeastern Australia. It employs a unique multivariate approach by assessing a complex suite of

performance and ecophysiological characteristics including photophysiological indicators (photosynthetic characteristics via PAM fluorometry and concentration of photosynthetic pigments), nutrient uptake dynamics (% C, % N and C:N ratios), C metabolism (stable C isotope $\delta^{13}C$), and RNA:DNA ratios (as a proxy for growth rate). These physiological performance indicators are assessed within the conceptual framework of the GRH, and ultimately enable evaluation of the suitability of bioclimate envelope models for predicting the future performance and distribution of seaweeds under projected climate change scenarios. The thesis also investigates the likely impacts of climate change-driven loss of seaweed canopy on associated understory communities, which is critical in evaluating the use of BEMs as the effects of biotic (competitive) interactions among species are normally assumed to be unimportant.

Chapter 2 investigates the influence of temperature and nitrate – two key environmental factors predicted to vary with future climate change – on the growth and ecophysiology of *P. comosa* from the northern and southern parts of its range. The physiological performance of this species was strongly temperature-dependent, but the deleterious effects of high temperatures on photosystem functionality were particularly pronounced in thalli originating from higher latitude. In contrast, nitrate availability played only a minor role in regulating seaweed physiology, suggesting that *P. comosa* at high latitudes may be less susceptible to nutrient stress than predicted by the GRH, but that the species will respond to predicted levels of warming with a loss of photosystem functionality unless rapid adaptation is possible.

Chapter 3 explores the extent to which *E. radiata* and *P. comosa* may acclimatise to environmental change through phenotypic plasticity, and how this plasticity may vary across their latitudinal distributions. Thalli were transplanted from low to high latitudes (with appropriate controls) and their physiological performance monitored over four months. Trait expression was found to be largely under environmental control despite considerable ecophysiological differences between *in situ* populations from sites at high and low latitudes, demonstrating the considerable capacity of these species to acclimatise to a wide range of environmental conditions.

Chapter 4 examines longer-term patterns in the seasonal *in situ* physiology of *E. radiata*, *P. comosa* and *M. pyrifera* in relation to environmental characteristics, and how this varies across their latitudinal and depth distributions. Temperature and irradiance were determined

to be important regulators of seaweed physiology, but as with controlled laboratory experiments, nitrate availability was less important (except for *M. pyrifera*, which has very limited internal nitrogen storage capacity). Seaweeds at high latitudes were more adversely affected by high temperature and low nutrients during summer than their lower latitude counterparts (particularly in shallower water) seemingly due to the synergistic interaction of climatic stressors with high irradiance. Thus, while predicted climate-driven changes may impact similarly on seaweeds across their latitudinal distributions, high latitude populations are likely to experience increasingly stressful summers and may retreat to deeper waters and/or become more sparse and patchy.

Chapter 5 assesses the impacts of potential climate change-driven thinning of seaweed canopy on the structure of associated understory community assemblages. Canopy thinning of *E. radiata* (as opposed to total canopy removal) affected a shift towards a foliose algal-dominated understory, with an associated loss of sponges, bryozoans and encrusting algae. While the structure of kelp-associated understory assemblages in southeastern Tasmania appears relatively stable, even partial loss of kelp canopy cover under future climate change scenarios will likely shift these communities towards a foliose algal-dominated state, which has important biodiversity implications.

This thesis demonstrates the considerable capacity of seaweeds to respond to environmental change through phenotypic plasticity. While no evidence was found to support the GRH, multivariate ecophysiological measurements revealed subtle latitudinal variation in seaweed physiology *in situ*; indicating that a broad-scale climate envelope approach will not adequately predict the future performance and distribution of seaweeds under predicted climate change. The key findings of this thesis are: (1) a multivariate approach is required to describe and interpret seaweed physiology, particularly when attempting to detect organismal-scale changes that may ultimately impact on the performance and local survival of a species; (2) the GRH does not apply to *E. radiata* and *P. comosa*, but seaweeds at higher latitudes are more susceptible to increasing water temperature than is often assumed due to the synergistic interaction between high temperature and high irradiance over summer; and (3) bioclimate envelope models are unlikely to be a useful tool for predicting the performance and distribution of seaweeds under future climate change scenarios.

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Statement of co-author contributions

Chapters 2-6 of this thesis have been prepared as scientific manuscripts for submission to peer-reviewed journals. In all cases the design and implementation of the research, data analysis, interpretation of results and manuscript preparation was the responsibility of the candidate but was carried out in consultation with supervisors and other specialist contributors. These contributions are outlined for each chapter below.

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Chapter 1 Introduction

Climate is the most important determinant of photosynthetic species distributions throughout terrestrial (e.g. Walter 1973, Woodward 1990) and aquatic (Santelices & Marquet 1998, Wernberg et al. 2010) environments worldwide, and the documented examples of climate change-driven shifts in species' ranges towards higher latitudes and altitudes continues to grow (e.g. Parmesan & Yohe 2003, Root et al. 2003, Perry et al. 2005, Wernberg et al. 2011a). Given the importance of the physical environment in regulating virtually every aspect of an organism's physiology, it is not surprising that the effects of changing climate will also dramatically impact key phenological and life history traits to permit the occupation of novel niches in newly-inhabited environments (e.g. Winn & Gross 1993, Zhang & M.J. 1994, Li et al. 1998, Bertness & Ewanchuk 2002, Santamaría et al. 2003). The physiological adjustments that organisms must make to compensate for environmental change may erode the resilience of populations and reduce their capacity to withstand multiple perturbations caused by natural or anthropogenic stressors, ultimately resulting in a loss of ecological function (Steneck et al. 2002, Folke et al. 2004, Wernberg et al. 2010). Understanding how global change will impact the distribution, abundance and performance of organisms is therefore of fundamental importance for effective management of ecosystems and conservation of biodiversity (Hannah et al. 2002), particularly given the unprecedented levels and rates of anthropogenic disturbance currently affecting natural systems (see review by Vitousek et al. 1997).

Studies on the likely impacts of environmental change on the distribution and performance of organisms have typically taken the form of controlled laboratory experiments in which a single environmental variable (e.g. temperature, CO_2, irradiance) is manipulated in isolation to determine changes in organismal performance (e.g. Dawes et al. 1998, Altamirano et al. 2004, Hoffmann 2010). While these studies are important to determine the physiological responses of a species to individual climatic factors (and thus understand the mechanisms of organismal-scale changes), they are not necessarily useful in predicting how a population will respond in the natural environment where individuals are exposed to multiple environmental

stressors. These may act additively, synergistically or antagonistically to produce non-linear effects that are not always predictable by single factor studies (e.g. Lotze & Worm 2002, Harley et al. 2006, Russell et al. 2009, Gaitán-Espitia et al. 2014). Determining the nature of interactions between multiple environmental stressors is critical to properly link predicted climate change and anthropogenic stressors with range shifts and the future ecological performance of biota (Crain et al. 2008, Darling & Côté 2008).

Bioclimate envelope modelling

Predictions of the likely impacts of climate change on natural systems from a spatially explicit perspective have largely focused around the use of bioclimate envelope models (BEMs) (e.g. Austin 2002, Berry et al. 2002, Araújo et al. 2005, Guisan & Thuiller 2005, Araújo & Luoto 2007). These empirical models relate field observations of a species' current geographic distribution with environmental predictor variables to forecast its future distribution into a new or modified habitat (Woodin et al. 2013). While BEMs are typically used to forecast future changes in distribution, they can also be validated using fossil records to hindcast a species' distribution from its present-day geographic occupation (e.g. Lawing & Polly 2011, Macias-Fauria & Willis 2013). The BEM concept relies on the fundamental assumption that models developed in one portion of a species range can be extrapolated, either in space or time, to the new conditions. This can be broken down into three main assumptions: (1) that predictions based on environmental conditions in one location will be valid in other locations with the same environmental conditions at other times, i.e. no evolutionary shifts occur in the characteristics of the species; (2) an organism's distribution is determined only by climate variables, and biotic interactions are not a limiting factor; and (3) the mechanism that limits the distribution of a species at the rear edge of its range (i.e. low latitude) will also be limiting to populations in other parts of its range when exposed to the same climatic conditions, i.e. consistency in the fundamental niche (Broennimann et al. 2007, Fitzpatrick et al. 2007).

While BEMs can, in some cases, provide a useful first approximation of the new distribution and/or performance of a species following environmental change (see review by Pearson & Dawson 2003), the approach has been criticised because the assumptions underpinning the models are not always ecologically plausible (Davis et al. 1998a, Davis et al. 1998b). There are a number of examples where BEMs have failed to accurately forecast a species' spatial occupation within a new habitat due to violation of one or more of these fundamental

assumptions (e.g. Beale et al. 2008, Beaumont et al. 2009, Roberts & Hamann 2012). Common causes of violation may include miscalculation of the fundamental niche (Beaumont et al. 2009), niche conservation failure (i.e. emergence of new niche space or contraction of an existing niche; Roberts & Hamann 2012), or strong biotic interactions (predation, competition or mutualism) – a factor neglected by BEMs (Brown et al. 1996, Beale et al. 2008). Moreover, the BEM concept typically projects the future range limits of a species based on the climatic tolerance of current rear edge populations (Nilsson-Örtman et al. 2012). In reality, populations from one portion of a species' range are usually highly specialised to perform in environments that may be unsuitable for populations from other parts of the range. For the traits of rear edge populations to apply to leading edge populations, peripheral populations would need to move along the species' distribution and outcompete previously established conspecifics without experiencing any changes to their genetic makeup or environmental tolerances (Hampe 2004). While this may be possible for highly motile organisms or those with high dispersal capabilities, a more realistic scenario is for extinction of rear edge populations, resulting in an overall reduction in a species' environmental tolerance.

The Growth Rate Hypothesis

One of the major factors affecting the physiological performance of an autotroph at any given point along a latitudinal (and environmental) gradient is its capacity to maintain tissue C:N:P in stoichiometrically balanced ratios (Sterner & Elser 2002). Either phosphorus (P) or nitrogen (N) can limit the growth and reproduction of autotrophs in freshwater (Hecky & Kilham 1988, Elser et al. 1990), marine (Hecky & Kilham 1988, Vitousek & Howarth 1991) and terrestrial (Walker & Syers 1976, Vitousek & Howarth 1991) environments, with the primary limiting nutrient dependant on a diverse set of geochemical and ecological factors. The Growth Rate Hypothesis (GRH), developed by Elser et al. (2003), proposes that autotrophs with higher growth rates will be selected for at higher latitudes to compensate for a growth season that is shortened by low temperatures and reduced light availability. As organisms maintaining high growth rates require high cellular concentrations of ribosomes, the GRH also predicts that rapidly-growing organisms will have low C:P and N:P ratios due to the increased synthesis of P-rich ribosomal RNA (Elser et al. 2000, Sterner & Elser 2002). A direct consequence is that organisms at high latitudes will have an increased susceptibility to nutrient limitation due to the greater demand placed on resources by rapid growth under favourable conditions (Kerkhoff et al. 2005, Lovelock et al. 2007). The GRH was originally

developed for terrestrial systems in which P is typically the limiting nutrient (due to high sequestration of P in soils; Walker & Syers 1976, Smith 1984). Phosphorus is less likely to limit the growth of marine organisms (particularly in temperate systems; Hurd et al. 2014) as oceanic systems are generally N-limited (Howarth 1988). While the GRH prediction of selection for higher growth rates at high latitudes may still apply to marine systems, this has yet to be rigorously tested.

In the context of predicting the effects of climate change on an organism's performance and distribution, the GRH conflicts with the fundamental assumptions of the bioclimate envelope concept in that it predicts physiological variation along a latitudinal gradient. This is particularly important in marine systems, where climate change and anthropogenic activities are predicted to alter the nutrient loading of coastal waters (which may take the form of oligotrophy or eutrophication depending on the spatial context and proximity to urban centres; e.g. Connell 2007, Rabalais et al. 2009, c.f. Ridgway 2007). For example, while coastal upwelling is predicted to intensify in some regions due to increased wind stress acting on the ocean surface (Bakun 1990), warming sea surface temperatures will strengthen thermoclines and reduce nutrient-rich upwelling (Roemmich & McGowan 1995, Doney et al. 2012), and climate-driven changes in the strength and/or trajectory of oceanic currents will (in some regions) drive incursions of nutrient-depleted waters into previously productive coastal ecosystems (e.g. Ridgway 2007). Thus, under the predictions of the GRH, marine algae at high latitudes will be more adversely affected by a given decline in nutrient availability than will their low-latitude conspecifics due to the strong selective pressure on higher growth rates. This violates the BEM assumption of fundamental niche consistency, as the mechanism(s) limiting growth will vary across an algae's distributional range. Understanding whether the GRH applies to a given ecological system is critical to (1) predict the way in which environmental change will impact on a species across its distribution; and (2) evaluate the utility of BEMs as a predictive tool for that system. Incorrect predictions of future species distributions can, in a conservation sense, be more dangerous than an absence of data, thus a mechanistic understanding of climate-regulated physiology and (for autotrophs) latitudinal variation in light-regulated photophysiology, should be considered an essential first step in the BEM process.

Effects of climate change on ecosystem engineering species

The velocity of climate change (geographic shifts in isotherms over time) is currently occurring at a similar rate across marine and terrestrial systems (Burrows et al. 2011). However, climate-driven shifts in species distributions are taking place at up to an order of magnitude faster in the marine environment than in terrestrial systems (Poloczanska et al. 2013). The rapid shifts in the distribution of marine species may be (at least in part) due to their wide-scale dispersal of propagules facilitated by ocean currents (Kinlan & Gaines 2003). However, sessile species such as benthic algae typically have very limited dispersal capacity (Coleman et al. 2009) and yet continue to retreat poleward at an alarming rate (Wernberg et al. 2011a). This is likely a result of multiple (potentially synergistic) anthropogenic-mediated stressors including altered ocean chemistry from increasing CO_2 uptake (Hoegh-Guldberg & Bruno 2010) and ecosystem-level disturbances resulting from heavy fishing pressure (e.g. Ling 2008). Range shifts involving keystone organisms such as biogenic habitat-forming seaweeds are particularly important, as these species provide the ecological foundations of most temperate rocky reef ecosystems (Steneck & Johnson 2013) and any changes in their performance or distribution has disproportionately large downstream effects on associated organisms and ecosystems (Dayton 1985, Marzinelli et al. 2013, Steneck & Johnson 2013).

The case study

Southeastern Australia is a global hotspot for seaweed diversity, with over 350 algal genera identified throughout the region (Kerswell 2006). This coastline is also home to some of the most productive seaweed beds in the world, and is dominated by the large canopy-forming phaeophytes *Ecklonia radiata, Macrocystis pyrifera* (Laminariales), *Durvillaea potatorum* and *Phyllospora comosa* (Fucales) (Kerswell 2006). Water temperatures in this region are increasing at a rate nearly four times the global average (Ridgway 2007), driven by increased wind stress in the Southern Ocean resulting in larger and more frequent incursions of warm East Australian Current (EAC) water – largely as eddies – off eastern Tasmania (Cai 2006, Ridgway 2007). The EAC water is nutrient-poor relative to sub-Antarctic water that has previously dominated oceanographic signatures in this region (Harris et al. 1987, Ridgway 2007), with summer nitrate levels typically < 0.5 µM and often not detectable. Consequently, ocean warming off eastern Tasmania is confounded with a decrease in nutrient loading, such that seaweeds in this region are exposed to multiple stressors simultaneously.

Given the recorded increases in ocean temperature of nearly 2.3 °C off southeastern Australia over the past 60 years (Ridgway 2007), it is not surprising that changes in the range, physiological performance, and competitive interactions of seaweed species have already been documented in this region. Herbarium records suggest that the distribution of the habitat-forming species *E. radiata*, *P. comosa* and *D. potatorum* have shifted southwards on the east coast of Australia over the past few decades (Millar 2007). For the laminarian *E. radiata* (one of the key species in this thesis), this represents a modest range shift relative to the species' extensive depth (0-60+ m) and latitudinal (27.5-43.5 °S) distribution throughout warm-temperate South Africa (Field et al. 1980), Australia (Steinberg & Kendrick 1999), New Zealand (Choat & Schiel 1982) and Japan (Bolton & Anderson 1994). However, the monotypic fucoid *P. comosa* (the second key species in this thesis) is restricted exclusively to the shallow high wave energy zone in southeastern Australia from Robe, South Australia, around Tasmania, and northward on the east coast to Port Macquarie, New South Wales (Campbell et al. 2014), thus a poleward range shift in southeastern Australia represents a significant range contraction for this species. In addition, seemingly permanent discontinuities in the distribution of both *P. comosa* and *E. radiata* have formed along large stretches of urbanised coastline in Sydney (Coleman et al. 2008, Coleman & Kelaher 2009) and Adelaide (Connell et al. 2008), respectively. However, perhaps the most visible decline in seaweed distribution in southeastern Australia has been that of the surface canopy-forming laminarian *Macrocystis pyrifera* (the third key species examined in this thesis). While the coverage of *M. pyrifera* has decreased in several regions across its latitudinal distribution (encompassing Alaska to California, South America, South Africa, New Zealand and southern Australia; Jackson 1982, Santelices & Ojeda 1984, Ladah et al. 1999, Nelson 2013), its areal coverage has declined by more than 95% in southeastern Australia over the past few decades such that the species is now restricted almost exclusively Tasmanian waters (Johnson et al. 2011). While the mechanisms responsible for this decline are currently under study, the most plausible cause is ocean warming and oligotrophy resulting from strengthening EAC influence (Johnson et al. 2011).

Given the capacity of seaweeds to acclimatise to annual cycles of temperature that are greater in range than the changes predicted by global warming scenarios (IPCC 2013), climate change is unlikely to lead to the complete removal of seaweeds from southeastern Australia. However, reduced resilience at the population scale may exacerbate other anthropogenic

perturbations and stressors, including climate-mediated diseases (Campbell et al. 2011), shifts in herbivore abundance (Ling 2008), climate-driven oligotrophy (Johnson et al. 2011) and eutrophy (Connell 2007), and increased frequency of severe storm events (Harley et al. 2006). Moreover, a permanent warming of 1-2 °C may be sufficient to exceed certain critical physiological thresholds resulting in sudden and catastrophic range shifts or contractions (Harley et al. 2012, Wernberg et al. 2013), as has been widely documented for other sessile marine species (see Scheffer et al. 2001 and references within). While seaweeds typically have general-purpose genotypes that can maintain high fitness over a broad range of environmental conditions through morphological (Wernberg et al. 2003, Wernberg & Thomsen 2005, Fowler-Walker et al. 2006, Stewart 2006) and/or physiological (Steneck et al. 2002, Wernberg et al. 2010) adjustments, this may come at a reduced fitness cost to individuals. Understanding the potential ecological costs of future environmental change to seaweeds, and how this may affect associated species and communities, is paramount to most effectively adapt to predicted climate change (Morton et al. 2009).

Thesis structure

This thesis examines ecophysiological aspects of the potential impacts of climate change on the important habitat-forming seaweeds *Ecklonia radiata, Phyllospora comosa,* and *Macrocystis pyrifera* in southeastern Australia. All aspects of the work are designed to inform an assessment of Elser's Growth Rate Hypothesis as it applies to this system, and thus ultimately to assess the suitability of standard bioclimate envelope methodology to predict the future performance and distribution of seaweeds under projected climate change scenarios.

The thesis begins by investigating the effects of two key environmental drivers (temperature and nitrate) on seaweed physiology in a controlled laboratory environment using *Phyllospora comosa* as a test species. It then explores the relationship between environmental conditions and the physiology of seaweeds *in situ* across their latitudinal and depth range in southeastern Australia, and investigates the role of physiological acclimation in influencing the success of seaweeds exposed to novel environmental conditions. It examines the applicability of the GRH to seaweeds in this system, and how this may result in differing impacts of environmental change across a species' latitudinal and depth distribution. The final element of the work investigates the consequences of climate change-driven loss of seaweed canopy for the structure and biodiversity of associated understory communities. This is an important component of the broader question because it focuses on the effects of biotic interactions

(competition) among species which, in applying BEMs to the questions of distribution and relative abundances under changed climatic conditions, is normally assumed to be unimportant.

Note that because this thesis has been prepared as a series of stand-alone manuscripts, repetition of important contextual information across the General Introduction (Chapter 1) and in the introductions of other chapters, and of methodological information across Chapters 2-4, has been unavoidable.

Chapter 2 outlines a laboratory experiment that explores the effects of temperature and nitrate availability within the ranges predicted by climate change scenarios (current temperature and [NO_3^-], increased temperature and depleted [NO_3^-]) on the physiological performance of *Phyllospora comosa*. The individual and interactive effects of these two key environmental drivers are explored, and the potential for latitudinal variation in their effects on seaweed physiology is discussed. A mechanistic explanation for the way in which temperature and nitrate availability influence *P. comosa* performance, and how this may apply to *in situ* populations subject to environmental change, is suggested.

Chapters 3 and 4 examine the physiological responses of seaweeds *in situ* to seasonally changing environmental conditions. Chapter 3 describes a latitudinal transplant experiment that investigates the influence of environmental factors in determining the expression of physiological traits in *E. radiata* and *P. comosa* (and thus the role of environmental conditions in regulating physiological performance), and explores the extent to which seaweeds can acclimate through phenotypic plasticity to novel environmental conditions. Longer-term patterns in seasonal physiology are further explored in Chapter 4, where the performance of *E. radiata, P. comosa* and *M. pyrifera* is measured using a number of physiological indicators and related to environmental characteristics across the latitudinal and depth distributions of the three species.

Chapter 5 investigates the impacts of potential climate change-driven thinning of *Ecklonia radiata* on the structure of associated understory community assemblages. It explores the importance of canopy density (or proportion of canopy loss) in determining community scale effects, and how this may influence future kelp recruitment and the structure of understorey assemblages under projected climate change scenarios.

In summarising, Chapter 6 provides a general discussion of the collective research and its context in the current body of seaweed ecophysiological literature. It explores the use of physiological metrics, both in this thesis and in the wider literature, as a proxy for direct measurements of growth and productivity, and highlights some of the issues associated with extrapolating physiological data to infer ecological conclusions. Results from this thesis collectively indicate that while the Growth Rate Hypothesis appears not to apply for *E. radiata* and *P. comosa* in southeastern Australia, broad-scale predictive approaches such as BEMs are unlikely to adequately account for biogeographic variation in seaweed physiology resulting from genetic differentiation across a species' distribution (Coleman et al. 2009, Coleman & Kelaher 2009), or spatial variability in the interactions between multiple environmental stressors. Hence, unless additional biological detail (such as latitudinal variation in thermal tolerances, genetic differentiation, and the mechanisms underpinning regulation of physiology by abiotic environmental factors) can be incorporated into the current ecophysiological modelling framework, BEMs are unlikely to accurately predict the impacts of future climate change on habitat-forming seaweeds.

Chapter 2 Phenotypic plasticity and biogeographic variation in physiology of a habitat-forming seaweed: response to temperature and nitrate

2.1 Abstract

The habitat-structuring fucoid *Phyllospora comosa* dominates shallow high wave energy zones on rocky substratum throughout southeastern Australia. This region is warming at nearly four times the global average rate due to seasonal incursions of warm, oligotrophic East Australian Current water. Under the predictions of the Growth Rate Hypothesis (GRH), simultaneous warming and oligotrophy will impact more severely on seaweeds with increasing latitude as growth seasons are shortened by light availability. Latitudinal variation in temperature and nutrient effects on the ecophysiology of *P. comosa* from northern and southern populations was investigated in a laboratory experiment using realistic temperature (12, 17, 22 °C) and nutrient (0.5, 1.0, 3.0 µM NO_3^-) scenarios. Changes in growth, photosynthetic characteristics (via chlorophyll fluorescence), pigment content, tissue chemistry ($\delta^{13}C$, % C, % N, C:N) and nucleic acid characteristics (absolute RNA and DNA, RNA:DNA ratios) were determined over 28 days. Performance of *P. comosa* was largely unaffected by nitrate availability but was strongly temperature-dependent, with photosynthetic efficiency, growth and survival significantly impaired at 22 °C. High latitude seaweeds were impacted more severely by elevated temperatures indicating a reduced capacity for thermal acclimation with increasing latitude. While some physiological processes (photosynthesis, nucleic acid and accessory pigment synthesis) responded rapidly to temperature, others (C and N dynamics, carbon concentrating processes) were largely invariant and latitudinal differences in these characteristics may only occur through genetic adaptation. No link was detected between nutrient availability and RNA synthesis, or between RNA:DNA ratios and growth, and the GRH was not supported. While *P. comosa* at high latitudes may be less susceptible to nutrient stress than predicted by the GRH, warming water temperatures will have more deleterious effects on seaweed at high latitudes unless adaptation is possible.

2.2 Introduction

An important challenge in understanding the impacts of climate change on ecosystems is predicting the responses of species and communities to expected levels of change. This is particularly critical when considering foundation, or ecosystem engineering species, that play a fundamental role in determining community structure and ecological processes (Jones et al. 1994, Lawton 1994). In temperate marine environments, macroalgal beds provide the ecological foundations for most shallow reef ecosystems (Dayton 1985, Steneck & Johnson 2013). Climate change is predicted to increase the importance of ecosystem engineers in maintaining healthy ecosystems because of their capacity to mitigate environmental stressors (Bruno et al. 2003). As a result, macroalgae are likely to play an increasingly important role in buffering the effects of climate change on temperate reef communities (Russell & Connell 2005, Russell et al. 2009, Wernberg et al. 2011b).

Predicting the responses of macroalgae to environmental change requires understanding the factors that affect growth, survival, and physiological processes (reviewed by Hurd et al. 2014). Often, multiple environmental factors affect individuals in ways that are not predictable from single factor studies due to nonadditive (synergistic or antagonistic) effects (Lotze & Worm 2002). For example, ocean acidification and eutrophication can act synergistically to displace native coralline species (Russell et al. 2009), while desiccation and hyposalinity have a negative synergistic effect on intertidal macroalgae (Kim & Garbary 2007), and the effects of ocean acidification on early life stages of giant kelp has been shown to vary with temperature (Gaitán-Espitia et al. 2014). Furthermore, the effect of environmental controls on macroalgal performance depends on both the species and its biogeographic context. Several laminarian species (particularly those at the upper extent of their thermal tolerance) are negatively affected by relatively small increases in temperature (e.g. Gunnill 1985, Wernberg et al. 2010, Mohring et al. 2013b), while other species (e.g. *Sargassum muticum*) respond positively to increased water temperatures (Norton 1977). Moreover, the physiological performance of macroalgae varies with latitude due to acclimation and adaptation via compensatory mechanisms at various biochemical levels (Davison & Davidson 1987, Raven & Geider 2003, Wernberg et al. 2010).

In addition to the demonstrated adaptive capacity of macroalgae to adapt and acclimate to environmental change (Hurd et al. 2014), physiological performance is also predicted to vary

with environmental (and hence latitudinal) gradients. This is explained (at least in part) by the Growth Rate Hypothesis (GRH), developed by Elser et al. (2003), which predicts that autotrophs with higher growth rates will be selected for at higher latitudes to compensate for a growth season shortened by temperature and light availability. Under ecological stoichiometry, an autotroph's performance depends on its capacity to maintain tissue C:N:P in stoichiometrically balanced ratios (Sterner & Elser 2002). As the growth rates of organisms is dependent on the allocation of resources (primarily P and N) to ribosomal RNA, rapid growth should be associated with higher nutrient requirements due to an increased demand for protein synthesis (Elser et al. 2003). The GRH combines these ideas and predicts an increased susceptibility of autotrophs at high latitudes to nutrient limitation due to the increased demand placed on resources by rapid growth during shortened growth seasons (Kerkhoff et al. 2005, Lovelock et al. 2007). While this concept has been widely demonstrated for rapidly-growing microalgae with short generation times (e.g. Dortch et al. 1983, Lepp & Schmidt 1998), evidence for its applicability to large, (relatively) slow-growing macroalgae is considerably more limited (Reef et al. 2012).

Southeastern Australia is home to some of the most productive macroalgal beds in the world (de Bettignies et al. 2014). This coastline is also warming at a rate nearly four times the global average (Johnson et al. 2011), driven largely by stronger and more frequent incursions of warm East Australian Current (EAC) water off southeastern Australia (in particular, eastern Tasmania; Ridgway 2007). The EAC water is nutrient-poor, with summer nitrate levels typically < 0.5 µM and often not detectable, relative to the sub-Antarctic water masses that have, until recently (last 2-3 decades), dominated waters off the east coast of Tasmania (Harris et al. 1987, Ridgway 2007). Consequently, ocean warming off eastern Tasmania is confounded with a decrease in nutrient loading, such that canopy-forming brown seaweeds such as *Ecklonia radiata, Macrocystis pyrifera, Durvillaea potatorum* and *Phyllospora comosa* that dominate this region are exposed to multiple simultaneous stressors. While the laminarian *E. radiata* has received considerable attention in relation to climate change research (e.g. Staehr & Wernberg 2009, Mabin et al. 2013, Wernberg et al. 2013), the monotypic fucoid *P. comosa* (hereafter *Phyllospora*) remains comparatively understudied despite its critical role in maintaining biodiversity (Marzinelli et al. 2013) and its dominance of shallow high wave-energy zones from Robe, South Australia, around Tasmania, and northward on the east coast to Port Macquarie, New South Wales (Campbell et al. 2014). Under the GRH, nutrient depletion (particularly low nitrates; Hurd et al. 2014) – in

combination with other potentially synergistic climate-driven stressors including ocean acidification (Wernberg et al. 2011b) and extreme warming events (which have previously been associated with localised dieback of *P. comosa* and *E. radiata*; Valentine & Johnson 2004, Wernberg et al. 2013) – may have disproportionately severe effects on *Phyllospora* at its southern distribution limits.

The predicted increase in temperature and decreased nitrates in southeast Australian waters is likely to negatively impact on the physiological performance of *Phyllospora* across its distribution range, as has been documented for *M. pyrifera* in eastern Tasmania (Johnson et al. 2011). However, our current understanding of the combined effects of these environmental stressors on the physiology, productivity and survival of macroalgae, and how this may vary with latitude, is limited. Thus, understanding how the GRH applies to macroalgae in this warming oligotrophic system is key to predicting spatial variation in the future impacts of climate change on ecosystem-structuring seaweed beds and their associated reef assemblages (Marzinelli et al. 2013).We hypothesised that, under the GRH, *Phyllospora* from high latitudes (southern Tasmania) will be more sensitive to decreases in nutrient availability than their more northern counterparts (NSW), and will respond with a greater reduction in growth and physiological performance. We also hypothesised that the combined effects of ocean warming and reduced nutrient availability will act in a synergistic manner to negatively impact on the performance of *Phyllospora* across all latitudes. While ecophysiological studies with algae have traditionally utilised measurements of growth (linear extension, change in tissue weight; e.g. Wheeler & North 1980, Dawes et al. 1994, Altamirano et al. 2000) or productivity (oxygen evolution methods; e.g. Littler 1979, Hanelt et al. 1997, Fairhead & Cheshire 2004a) to relate performance to environmental conditions, single growth or productivity measures can fail to detect underlying physiological information that may be involved in organism-scale adaptive changes (Roleda et al. 2004). To test our hypotheses, we determined changes in a range of performance and ecophysiological traits (photosynthetic characteristics, pigment content, $\delta^{13}C$ values, carbon (C) and nitrogen (N) content, absolute RNA and DNA concentrations, RNA:DNA and C:N ratios) under manipulated temperature and nutrient conditions representing current and realistic future climate change scenarios. We also compared the performance of *Phyllospora* from the northern and southern parts of its range to assess potential latitudinal differences in its physiological response to predicted environmental change.

2.3 Materials and Methods

2.3.1 Study species, seaweed collection, and *in situ* sampling

Phyllospora comosa (Fucales) grows to ~0.5-3 m in length, with a short stipe bearing multiple primary branches. *Phyllospora* dominates the shallow high-wave energy zone on rocky reefs in south-eastern Australia, typically excluding other canopy-forming species to form a monospecific band from 0-5 m depth but extending below ~10 m on exposed coasts (Coleman et al. 2008). Juvenile *Phyllospora* were collected in February 2012 from a depth of 5-6 m on exposed rocky coast near its northern range limit at Nelson Bay, NSW (32.791° S, 152.107° E) and near its southern range limit at Fortescue Bay, Tasmania (43.123° S, 147.976° E). Ocean temperatures measured *in situ* at the time of seaweed collection were ~23 °C (NSW) and ~17 °C (Tasmania) and are close to the maximum temperatures for these regions. Attempts were made to collect thalli of a similar size, with a blade length of 60-100 mm. Immediately following collection, seaweeds were transferred to aerated filtered seawater and held under low light (12 µmol photons m^{-2} s^{-1}) and temperature (13-15 °C, monitored at 2-hourly intervals and maintained by addition freezer blocks to reduce water temperature when necessary) for 24 hours during which time they were transported to the laboratory. Pilot trials indicated that survival of *Phyllospora* was maximised by keeping individuals at low light and temperature immediately following field collection (even NSW thalli, which experienced a proportionally greater change in temperature) as rates of respiration and photosynthesis are slowed under these conditions (Peckol 1983) thus reducing the likelihood of resource (O_2, CO_2, nutrient) stress. Thalli from both locations were treated identically except that those collected in NSW were transported by air to Tasmania. Tasmanian thalli were held in darkness for 3 hours to replicate the complete darkness experienced by NSW thalli during the flight from NSW to Tasmania.

At the time of collection, baseline PAM fluorometric measurements were taken *in situ* from a randomly selected set of five individuals in each location, and tissue was collected from each for analysis of pigment content, tissue chemistry, and nucleic acids. Tissue was collected by removing a single lateral from thalli *in situ* using gloved hands and sealing in lightproof containers which were brought to the surface. Tissue was removed from containers at the surface but maintained under low light and rinsed with de-ionized water before being cut into three pieces for separate analyses (see section 2.3.3 *Ecophysiological measurements* for

further details on all sampling techniques described above). All samples were kept on ice in darkness for transport immediately following collection and were refrigerated or frozen (as required) within six hours of collection.

2.3.2 Experimental design and culture conditions

The effects of temperature and nitrate on *Phyllospora* physiology were explored in a laboratory experiment using a factorial three-way design with the factors latitude of origin (2 levels: NSW, Tasmania); nitrate concentration (3 levels: 0.5, 1.0, 3.0 µM NO_3^-); and temperature (3 levels: 12, 17, 22 °C). Seaweeds were grown under treatments for 28 days with $n = 3$ independent replicates for each combination of temperature, nitrate and site of origin. As our primary focus was on the effects of temperature and nitrates on *Phyllospora* growth and physiology, seaweeds were only sampled from a single site in each region (NSW or Tasmania) due to the large number of measurements required by the factorial design.

Following collection, juvenile seaweeds were established in 2000 ml glass beakers where they were gradually and incrementally acclimated to experimental conditions to minimise potential stress resulting from sudden environmental changes. Initial transport conditions (13–15 °C, 12 µmol photons m^{-2} s^{-1}) were increased or decreased as required over a period of four days to the desired experimental irradiance (30 µmol photons m^{-2} s^{-1}) and temperature treatments, with irradiance increased in daily steps of ~10 µmol photons m^{-2} s^{-1} and temperature increased or decreased by ~2 °C per day. Once the desired treatments were reached for all thalli, conditions were maintained for a further 2 days (6 days total acclimation time) which is comparable with acclimation times in other similar seaweed studies (e.g. Orfanidis & Haritonidis 1996). Thalli were orientated vertically but were not attached to substratum, as pilot trials had indicated that these seaweeds were unlikely to attach to artificial or natural substratum over the duration of the experimental period, and the physiological performance of attached thalli was not measurably different to free-floating individuals. Growth media was changed every 2–3 days over this acclimation period, and again immediately before commencement of the experiment to ensure fresh nutrient supply.

Growth media (Wright's Chu #10 media; Guillard & Lorenzen 1972, reviewed by Andersen 2005) was prepared using fresh filtered oceanic seawater and augmented with sodium nitrate ($NaNO_3$) to achieve nitrate concentrations representing the range of normal (3.0 µM NO_3^-)

and depleted (< 0.5 µM NO_3^-, summer EAC influence) levels of nitrate recorded off Maria Island, eastern Tasmania (Rochford 1984). Depleted NO_3^- levels are also representative of coastal NSW waters which are exposed to EAC influence year-round (Rochford 1984). Although NH_3 and NO_2^- are also known to be utilised by seaweeds as an inorganic N source (Pedersen & Borum 1996, Ahn et al. 1998), concentrations of NO_3^- at the NSW and Tasmanian sites sampled throughout the seasonal cycle were typically double that of NH_3 and NO_2^- combined (Chapter 4), hence NO_3^- was used as the N source in the growth media.

Nitrogen was the element of interest for manipulation in this study as it is typically the primary element that limits seaweed growth in temperate systems (Hurd et al. 2014), but phosphorous levels were also manipulated in the form of potassium hydrogen phosphate (K_2HPO_4) to maintain a stoichiometrically balanced N:P ratio of 20:1 (Guillard & Lorenzen 1972). Silicates were omitted from the media to eliminate the need for germanium dioxide (a suppressor of diatom cell division) because of its potentially inhibitory effects on growth of juvenile macroalgae (Shea & Chopin 2007). Media was prepared using fresh filtered oceanic seawater as the base component in 20 L carboy containers and was stored in darkness for no more than 24 hours before use. Background levels of NO_3^- in seawater were measured before media preparation using flow injection analysis (Analytical Services Tasmania) and were undetectably low (i.e. < 0.15 µM) at the time of use, so added NO_3^- was assumed to account for the total NO_3^- levels in experimental treatments. Media in beakers was changed every 5 days to prevent depletion of nutrients and reduce the likelihood of bacterial contamination.

Temperature was manipulated using temperature-controlled rooms set at 12, 17 and 22 °C. This range of temperatures was selected because it approximated the range of temperatures experienced by *Phyllospora* at the sites in NSW and Tasmania (Tasmanian winter mimina ≈ 11 °C, NSW summer maxima ≈ 23 °C; Chapter 4). Each room was maintained at a light environment of 30 µmol photons m^{-2} s^{-1} on a 12 h light : 12 h dark cycle. This level of irradiance was chosen based on *in situ* irradiance data from beneath the *Phyllospora* canopy in Tasmania which ranged from 16-66 µmol photons m^{-2} s^{-1} (mean midday PAR measured at 5 m over a 2 week period in February 2012; Flukes et al. *unpub. data*), and the results of pilot studies we conducted for this experiment which demonstrated significant bleaching of thalli within 7 days of exposure to laboratory irradiance ≥ 40 µmol photons m^{-2} s^{-1}. Lighting consisted of 36 W white fluorescent tubes, and the entire experimental area was enclosed in

black plastic to isolate treatments from external light. Each beaker was covered and aerated using an aquarium air pump with 4 mm silicon tubing passing air through a single terminal filter (Sartorius Stedum Biotech, Minisart single use syringe filter 0.2 µm) to minimise contact with unfiltered air. Aeration was maintained just below the water surface to ensure maximal water circulation without exposing seaweeds to potentially physically damaging bubbles.

2.3.3 Ecophysiological measurements

Ecophysiological measurements were taken from separate thalli at both the start (t = 0 d; ie. after the 6 day acclimation period) and end (t = 28 d) of the experiment. At t_0, each beaker contained two individuals that had been exposed to the same acclimation regime. One from each pair was sampled using PAM fluorometry (see more below) before being sacrificed for the full suite of initial ecophysiological measurements. For tissue chemistry (C, N and $\delta^{13}C$), 10-20 mm^2 of tissue was removed from each plant with tweezers and rinsed with de-ionized water before freezing immediately at -20 °C for mass spectrometric analysis. A similar sized piece of tissue was removed and stored in 2 ml of RNAlater (Ambion, Inc., Austin, TX), a nontoxic RNA stabilization buffer, for nucleic acid (RNA, DNA) measurements. These samples were refrigerated overnight at 4 °C before being transferred to -80 °C for long-term storage, in accordance with the recommendations of Ambion. A third section of tissue 20-40 mm^2 in size was stored in darkness at -20 °C for pigment analysis. The second thalli in each beaker was followed over 28 d and its growth determined before being sampled by PAM fluorometry and sacrificed for the same suite of ecophysiological measurements. In some cases where thalli became necrotic, these were destructively sampled, although this never occurred more than 5 days before the scheduled experimental end point and affected 4 and 5 of the 27 individuals from NSW and Tasmania, respectively (all subject to water at 22 °C). These data were treated in the same way as terminal measurements that were collected at the conclusion of the full experimental period, with relative growth rates corrected for the shortened growth period (see more below).

Growth rates

We did not use the hole-punch method (originally developed by Mann 1972) for measuring meristematic growth due to potential issues associated with infection and impairment of photosynthetic ability in these small juvenile thalli. Instead, we photographed whole thalli

and used the software ImageJ (v1.46) to measure total thallus area of individuals to the nearest mm at $t = 0$ and $t = 28$ days (or upon termination). Relative growth rates per day as a function of total thallus area were then calculated as 100[ln(final area) – ln(initial area)]/days, as per Kain and Jones (1976).

Elemental composition of tissue

Frozen algal samples were thawed, blotted dry and freeze dried for 18 hours before being ground and homogenised. Approximately 5 mg of each dried, powdered sample was weighed into tin cups (Elemental Microanalysis LTD., Okehampton, UK) which were closed prior to analysis. Carbon, nitrogen, and $\delta^{13}C$ and $\delta^{15}N$ content were determined using a Thermo Gas Chromatograph coupled to a Finnigan Mat Delta S isotope radio mass spectrometer operating in continuous flow mode (analysed by CSIRO, Hobart). Results are presented in standard sigma notation:

$$\delta^{13}\text{C or } \delta^{15}\text{N } (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \qquad \text{where R} = {}^{13}\text{C}/{}^{12}\text{C or } {}^{15}\text{N}/{}^{14}\text{N}$$

Externally validated working standards were replaced every 12 samples, with Pee Dee Belemnite (VPDB) used throughout as a standard for carbon and air as a standard for nitrogen. Within-sample variability from a subset of duplicate samples (>20% of total samples run) gave the variability for C, N, C:N, and $\delta^{13}C$ (respectively) as ± 0.68%, 0.05%, 0.59, and 0.32‰ (mean standard error of duplicate samples).

RNA and DNA determination

Absolute cellular concentrations of RNA and DNA (and RNA:DNA ratios) were used to determine the proportion of protein synthesis machinery per cell as a proxy for "growth potential" of thalli. RNA and DNA content were determined by extraction from a single tissue sample. Frozen samples stored in RNAlater were thawed, patted dry, and a 1-5 mg (wet weight) subsample was weighed and homogenised with a drill pestle in a solution containing 500 µL of urea (4 M), sodium dodecyl sulphate (SDS, 1%), trisodium citrate (1 mM), sodium chloride (0.2 M) and 5 µL of Proteinase K (A.R. Bridle *unpub. data*). To ensure complete cell lysis, digestion of RNAses by Proteinase K, and stabilisation of nucleic acids, the homogenised solution was incubated at 37 °C for 10 minutes before being placed on ice. 750 µL of ammonium acetate (7.5 M) was added to the cooled solution, vortexed and centrifuged

for 5 minutes at 14,000 RCF to precipitate impurities (e.g. chlorophyll, phenolic compounds, salts, detergents in urea/SDS, etc.). The resulting supernatant, containing total nucleic acid (tNA), was decanted into a 1.5 ml tube and precipitants discarded. 700 µL of isopropanol was added to the supernatant and tNA was pelletised by gently inverting the tube 40 times before centrifuging for 10 minutes at 14,00 RCF. The tNA pellet was washed twice in a 75% ethanol (EtOH) solution, resuspended in 200 µl molecular grade H_2O and split into two 100 µL aliquots.

A solution of 20 µL 10x DNase I reaction buffer (New England Biolabs – B0303S), 5µL DNase I (New England Biolabs - M0303L) and 80 µL molecular grade water was added to one aliquot of tNA suspension for total DNA digestion, and 5 µL of RNAse A (Sigma Aldrich - R6148) with 100 µL of molecular grade water was added to the other aliquot to digest total RNA. Both tubes were incubated at 37 °C for 20 minutes to facilitate digestion (as per supplier's recommendations) then stabilised on ice. Impurities were further precipitated by vortexing in 400 µL of a urea/SDS buffer followed by vortexing and centrifuging with 200 µL of ammonium acetate (7.5 M). Isopropanol was added to the two aliquots of supernatant and total RNA and DNA precipitated as per the previous step. RNA and DNA pellets were washed twice in 75% EtOH and resuspended into 100 µl of molecular grade water (RNA) or EB buffer (DNA). Final concentrations of RNA and DNA were quantified by fluorescence assays performed using a Quibit assay probe and fluorometer. Nucleic acid concentrations were expressed as total RNA and DNA (µg.g wet weight^{-1}) which were used to calculate RNA:DNA ratios. Within-sample variability from a subset of duplicate samples (>20% of total samples extracted) gave the variability for total RNA, total DNA, and RNA:DNA (respectively) as ± 16 µg.g ww^{-1}, 3.1 µg.g ww^{-1}, and 3.6 (mean standard error of duplicate samples).

Pigment extraction

Frozen samples collected for pigment extraction were thawed and blotted dry, then weighed to the nearest 0.01 mg. Approximately 100-150 mg (wet weight) of algal tissue was transferred to a 15 ml vial and extracted into 5 ml of N,N-dimethylformamid (DMF). Processing was conducted rapidly under low light to minimise sample deterioration or change in pigment content resulting from exposure to ambient light. Vials were wrapped in aluminium foil and left in total darkness for 96 hours at -20°C for extraction to take place. Tissue was white in colour following DMF extraction, indicating a complete removal of

chlorophyll. Following extraction, 2 ml of extractant was removed, centrifuged at 8,000 rpm for 8 minutes and frozen at -80 °C for High Performance Liquid Chromatography (HPLC) analysis.

Centrifuged samples for fucoxanthin determination were measured in 2 µl aliquots using a Waters Acquity H-series UPLC™ (Waters Corporation MA. USA) coupled to a Waters Acquity Photodiode Array Detector (PDA) with an Ethylene Bridged Hybrid (BEH) C-18 column (2.1 x 100 mm x 1.7 micron particles). The mobile phase was a gradient mixture prepared from Merck Chemicals (Merck KGaA, Darmstadt, Germany) with three eluents: 1% acetic acid, acetonitrile, and 80:20 methanol:hexane. Initial conditions were a mixture of 20% acetic acid and 80% acetonitrile for 3.5 minutes, followed by an immediate switch to 80% acetonitrile and 20% methanol:hexane which was then held for a further 5.5 minutes. Samples were left for 3 minutes and allowed to re-equilibrate. Initial calibration of the UV-Vis response at 440 nm for fucoxanthin was carried out on a fresh, accurately prepared standard solution (Sigma Aldrich, Castle Hill, Australia) made up at 1.26 µg mL^{-1} in methanol. The column was held at 35 °C with a flow rate of 0.35 mL $minute^{-1}$ throughout. Chromatograms were extracted at 440 nm from the raw data using Waters TargetLynx V4.1 software. The area beneath the fucoxanthin peak was recorded and converted to mg mL^{-1} fucoxanthin, then to mg g^{-1} wet weight.

Chlorophyll *a* and *c* content was measured from the remaining ~3 mL of DMF extracted sample using a Dynamica HALO RB-10 Spectrophotometer and processed using UV Detective software (v 1.1). Samples were spectrophotometrically measured at the wavelengths 664.5, 631, and 582 nm. Where absorbance was above 1.000, samples were diluted with DMF until readings dropped below 1.000 in accordance with the manufacturer's recommendations. DMF was used as a blank throughout.
The following equations were used to calculate pigment content (expressed as mg ml^{-1}):

$$[\text{Chlorophyll } a] = (12.7 * E_{\lambda 664.5}) / 1000 \quad \text{(Inskeep \& Bloom 1985)}$$

$$[\text{Chlorophyll } c] = (E_{\lambda 631} - E_{\lambda 582}) - (0.297 \times E_{\lambda 664.5}) / 61.8 \quad \text{(Seely et al. 1972)}$$

where E_x = the measured absorption coefficients at wavelength of *x* nm.

Pigment concentrations were then converted to mg g^{-1} wet weight.

Pulse amplitude modulated (PAM) fluorometry

Electron transport dynamics in photosystem II (PSII) were estimated by variable chlorophyll *a* fluorescence using a blue LED Diving-PAM (Walz, Germany). Prior to fluorescence measurements, epiphyte and bryozoan growth was wiped from the blade surface with gloved fingers. Algal thalli were connected to a "dark leaf clip" (as termed by Walz) to maintain a consistent spacing of the PAM fiber optic from the surface of the tissue and permit fluorescence measurements without ambient light interference. An inbuilt software routine was used to generate rapid light curves (RLCs), where actinic light intensity was increased in eight steps (reaching a maximum of 463 µmol photons m^{-2} s^{-1} at 650 nm) of 10 seconds in duration to measure the effective quantum yield of PSII (ϕ_{PSII}) as a function of irradiance. Relative electron transport rate (rETR) is an approximation of the rate of electrons pumped through the photosynthetic chain (Beer et al. 2001), and can be calculated as ϕ_{PSII} x PAR. Plotting rETR against PAR creates a curve similar to a photosynthesis-irradiance (P-E) curve (although it should not be interpreted as such; see Ralph & Gademann 2005) and provides an assessment of photosynthetic activity.

RLCs were conducted on 15–20 minute dark-acclimated tissue (determined by our pilot studies to be sufficient to allow oxidation of the electron transport chain and relaxation of photoprotective mechanisms; i.e. further dark-acclimation resulted in no further increases in F_m or F_v/F_m; Appendix A). While ambient light-acclimated RLCs demonstrate the thalli's immediate light history and can be affected by recent canopy shading, water clarity, cloud cover etc., dark-acclimation 'standardises' the pre-manipulation light history and reflects the inherent physiological state of photosystems beyond the immediate light history (Ralph & Gademann 2005). All PAM fluorometry was conducted between 12:00 and 14:00 to further minimise potential variability in photosynthetic activity due to diurnal cycles of non-photochemical quenching (e.g. Belshe et al. 2007). All RLCs were conducted on fresh tissue, with the leaf clip moved 20-30 mm between initiations of each RLC to avoid light stress affecting subsequent fluorometric measurements.

Curves were fitted to raw rETR data to derive the saturating light intensity (E_k) and maximum electron transport rate, or photosynthetic capacity ($rETR_{max}$). The maximum quantum yield, or intrinsic potential efficiency of PSII (F_v/F_m), was also derived from dark-acclimated samples (see Falkowski & Raven 1997). The double exponential decay model of

Platt et al (1980) was selected as the model fit because it adequately described both the initial linear response and the region of photoinhibition (Ralph & Gademann 2005):

$$P = P_s\left(1 - exp\left(\frac{-\alpha E_d}{P_s}\right)\right) \times exp\left(\frac{-\beta E_d}{P_s}\right)$$

where P is the photosynthetic rate (here used as rETR), α is the light harvesting efficiency as measured by the initial slope of the RLC before light saturation, E_d is the downwelling actinic irradiance of the PAM's internal halogen light, β is the negative slope of the RLC at high irradiance, and P_s is a scaling factor defined as the maximum potential rETR in the absence of any photoinhibition (if $\beta = 0$, this is equal to $rETR_{max}$). Nonlinear least-squares function using the 'R' software environment (v 3.0.0) was used to fit models, and the parameters $rETR_{max}$ (maximum relative electron transport rate) and E_k were estimated as per the equations given in Ralph & Gademann (2005). The following parameters were used in the curve-fitting routine to ensure convergence: iterations = 100; stepsize = 1/1024; tolerance = 0.00001; initial seed value for P = maximum rETR derived from raw data, α = slope of linear regression fitted to first three points of raw data (typically in the range 0.7 – 1.0).

2.3.4 Statistical analysis

Analysis of Variance (ANOVA) was used at both the start (t = 0 d), and end (t = 28 d), of the experiment to determine the significance of differences in photosynthetic parameters ($rETR_{max}$, E_k, F_v/F_m); tissue chemistry (% C, % N, C:N, and $\delta^{13}C$); RNA:DNA ratios; pigment concentrations (chlorophyll *a* and *c*, fucoxanthin); and absolute and relative growth rates (end only) of *Phyllospora* across the different treatments. A three-way factorial design was used, with the factors 'temperature', 'nitrate' and 'latitude of origin'. For all ANOVA, data were checked for conformity to the assumptions of homoscedasticity and normality of residuals. Where heteroscedastic, the transformations to stabilise variances were determined by the relationship between SDs and means of groups (Draper & Smith 1981). Log transformations were applied to the following variables to stabilise variance: E_k, chlorophyll *a*, chlorophyll *c*, RNA:DNA. Where factors had a significant effect on a dependent variable, *a posteriori* multiple comparisons were conducted using Tukey's HSD test. The 'baseline' *in situ* measurements of the same set of dependent variables in NSW and Tasmanian seaweeds were compared in separate analyses by use of two-tailed student's t-tests. Multivariate

Analysis of Variance (MANOVA) was also conducted on normalised response variables at both the start and end of experiment to identify the potential effect of treatments evident from the joint distributions of dependent variables not detectable by univariate ANOVA. The statistical package 'R' (v 3.0.0) was used for all analyses.

2.4 Results

Rapid Light Curves (RLCs) derived from PAM fluorometry showed identical patterns (i.e. treatment effects) for tissue where fluorescence was measured following 15-20 minutes of dark acclimation, and for seaweeds adapted to ambient light conditions (data not presented here). Consequently, only results from dark-acclimated tissue are presented, ensuring that PSII reaction centres are open and photoprotective mechanisms relaxed.

2.4.1 Baseline measurements (characterisation of inherent physiological differences)

Baseline measurements of *Phyllospora* taken at sites in NSW and Tasmania revealed a number of differences in *in situ* carbon acquisition, photophysiology, and pigment composition. Initial RNA:DNA ratios and tissue concentrations of C and N (and thus C:N ratios) were similar between seaweeds at Nelson Bay, NSW and Fortescue Bay, Tasmania; but NSW thalli had a higher (less negative) fraction of the heavy carbon isotope $\delta^{13}C$ ($p < 0.001$; Figs 2.1 & 2.2). Photosynthetic capacity ($rETR_{max}$) was significantly higher in seaweeds at the NSW site ($p = 0.008$; Fig. 2.3), as were tissue concentrations of chlorophyll *c* ($p = 0.024$) and fucoxanthin ($p = 0.015$; Fig. 2.4). NSW seaweeds contained slightly less chlorophyll *a* than Tasmanian individuals ($p = 0.046$; Fig. 2.4), and had more than double the ratio of chlorophyll *c* : chlorophyll *a* (NSW 0.23 ± 0.02, Tas 0.10 ± 0.01; mean ± SE).

2.4.2 Effects of acclimation

Several physiological measurements responded rapidly (over several days or less) during the acclimation period, whereas other metrics were unaffected. The pigment content and chemical characteristics of *Phyllospora* were largely unchanged from the *in situ* values following 6 days of acclimation (NSW seaweeds retained more chlorophyll *c*, fucoxanthin and a greater fraction of $\delta^{13}C$, while Tasmanian thalli retained more chlorophyll *a*; Table 2.1A; Figs 2.1 & 2.4). Ratios of RNA:DNA in NSW seaweeds declined from *in situ* values

with increasing treatment temperature (significant Temperature x Origin interaction; Table 2.1A; Fig. 2.2C), but remained consistent in Tasmanian thalli. In contrast, RLC characteristics changed rapidly with temperature (Table 2.1A; Fig. 2.3) such that seaweeds acclimated to different temperatures had vastly different photosynthetic characteristics at the start point of the experiment ($rETR_{max}$ and E_k were both highest at 22 °C, then 17 °C, and lowest at 12 °C), and the initial distinction in $rETR_{max}$ between NSW and Tasmania thalli rapidly disappeared (Table 2.1A; Fig. 2.3). Photosynthetic efficiency, (F_v/F_m), while not scaling with temperature in the same manner as $rETR_{max}$ and E_k, was lower at 22 °C relative to other temperature treatments following acclimation (Table 2.1A; Fig. 2.3).

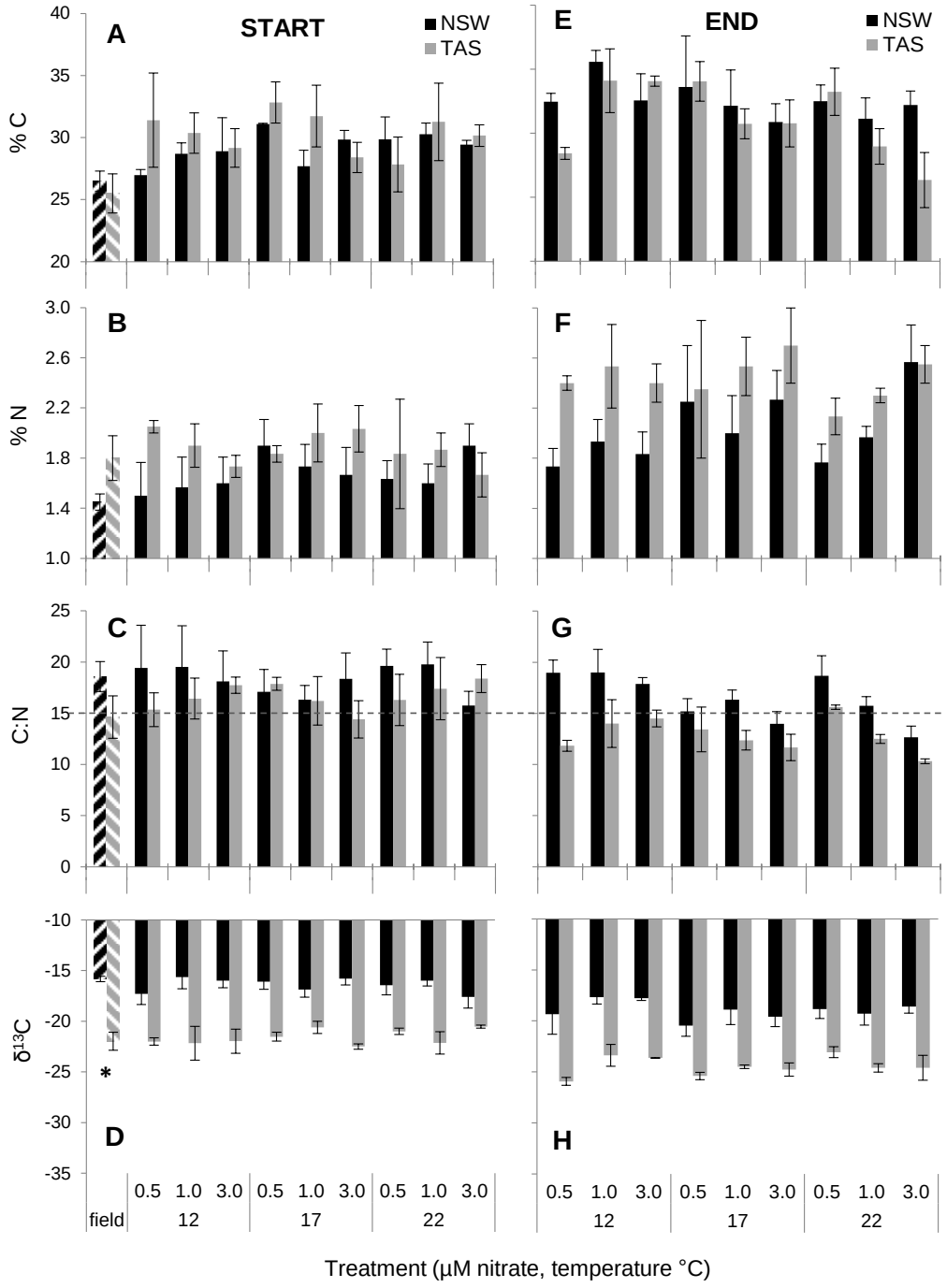


Figure 2.1 Changes in the chemical characteristics of juvenile *Phyllospora comosa* originating from NSW (black bars) and Tasmania (grey bars) grown under factorial combinations of temperature and nitrate concentrations. Plots show **(A, E)** % C; **(B, F)** % N; **(C, G)** C:N; and **(D, H)** δ^{13}C;. Values are shown from the **(A-D)** start (diagonal stripes show *in situ* field samples, with asterisks indicating student's t-test significance); and (**E-H)** end of a 28 day laboratory growth experiment. Dashed line in **C, G** indicates the approximate nitrogen limitation threshold for macroalgae (C:N ≥ 15; Lapointe 1987). All seaweeds were acclimated to 'start' conditions for 6 days before initial sampling took place. Data from thalli that were sacrificed prematurely due to necrosis (maximum 5 days early, at 22 ℃ only) have been included with other 'terminal' measurements. Bars indicate mean values (n = 3) ± SE.

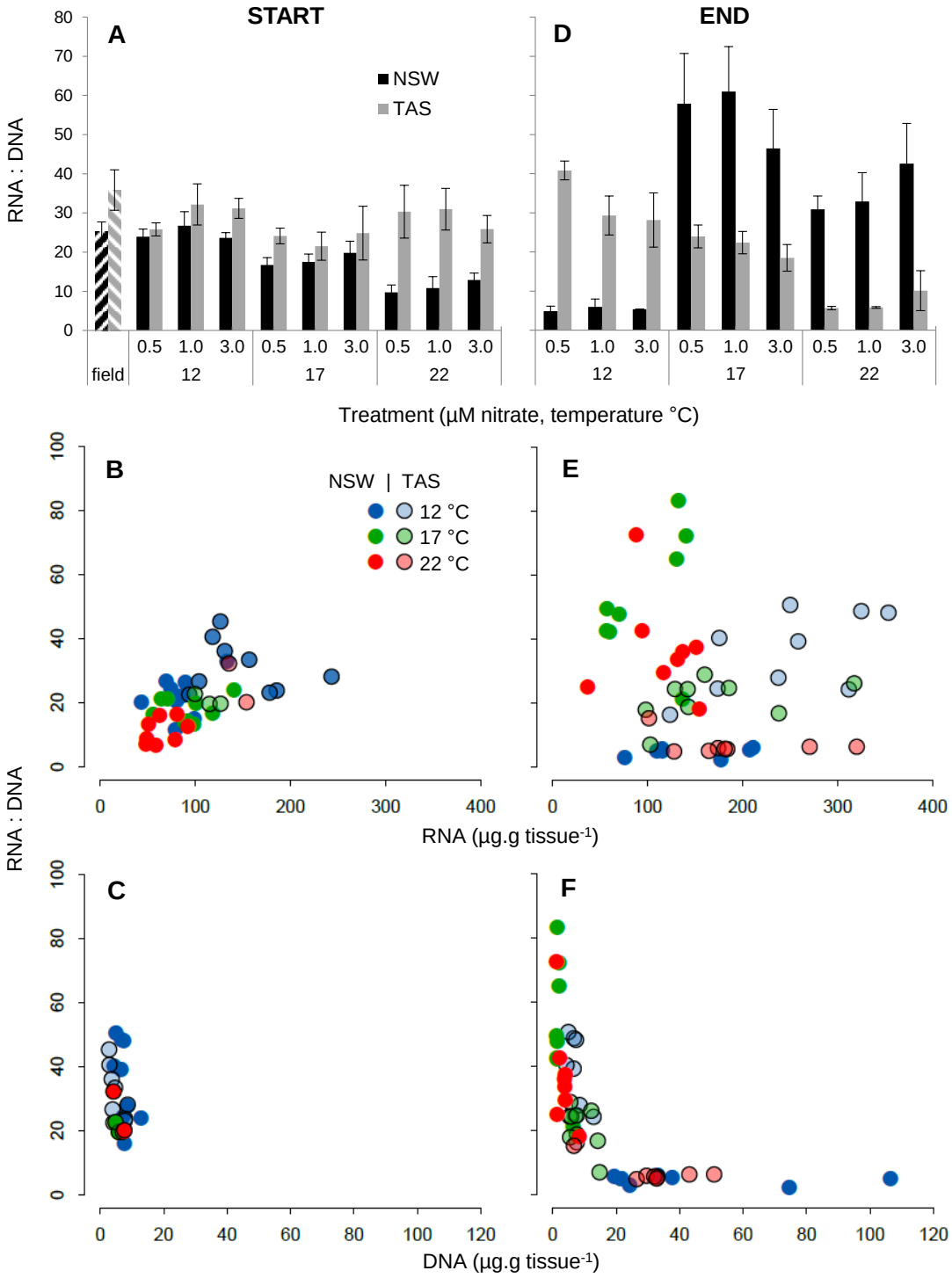


Figure 2.2 Changes in the nucleic acid content of juvenile *Phyllospora comosa* originating from NSW (black bars, solid borderless points) and Tasmania (grey bars, translucent bordered points) before **(A-C)** and after **(D-F)** 28 days of growth under manipulated temperature and nutrient conditions. Plots show **(A, D)** mean RNA:DNA ratios under factorial combinations of temperature and nitrate; **(B, E)** absolute RNA:DNA *vs.* absolute RNA, and **(C, F)** absolute RNA:DNA *vs.* absolute DNA. Colours in scatter plots indicate temperature treatments. Note that absolute RNA and DNA concentrations were not available for several Tasmanian individuals at 17 and 22 °C the start of the experiment **(B, C)**. Bars plots show mean values (n = 3) ± SE, with striped bars indicating *in situ* field samples. All seaweeds were acclimated to 'start' conditions for 6 days before initial sampling took place.

Table 2.1 Three-way factorial ANOVA testing the effect of nitrate (3 levels), temperature (3 levels), and latitude of origin (2 levels), on photosynthetic characteristics, pigments, tissue chemistry, nucleic acid ratios, and growth of *Phyllospora comosa* at the **(A)** start; and **(B)** end of a 28 day laboratory growth experiment. Results of Tukey's HSD tests for significant results are given below table. Abbreviations: chlorophyll *a* (chl *a*), chlorophyll *c* (chl *c*), fucoxanthin (fucox), relative growth rate (RGR). All seaweeds were acclimated to 'start' conditions for 6 days before initial sampling took place. Only degrees of freedom (*df*), *F*-values and significance levels are shown (error *df* = 36 for all tests). Significant tests are shown in bold. *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

A		Photosynthetic characteristics			Pigments			Tissue chemistry				Nucleic acids
Factor	*df*	$rETR_{max}$	E_k	F_v/F_m	Chl *a*	Chl *c*	Fucox	% C	% N	C:N	$\delta^{13}C$	RNA:DNA
Temperature (T)	2	**103*** **	**131*** **	**11.5** **	1.28	0.73	0.71	0.68	1.08	0.35	0.51	**7.14** **
Nitrate (N)	2	0.54	0.09	0.35	0.22	0.41	0.14	0.30	0.03	0.04	0.27	0.33
Origin (O)	1	0.16	1.02	0.08	**18.4*** **	**18.6*** **	**7.26***	2.06	3.46	1.15	**160*** **	**29.3** **
T x N	4	0.30	0.44	0.56	0.95	0.21	1.24	1.07	0.11	0.25	0.28	0.44
T x O	2	2.21	1.80	1.21	2.03	0.09	0.48	0.68	0.38	0.17	0.70	**6.02** **
N x O	2	0.32	0.35	0.38	2.46	1.29	1.29	0.75	0.51	0.10	0.16	0.10
T x N x O	4	0.20	0.19	0.12	0.64	0.20	0.67	0.94	0.86	0.77	1.84	0.49
Tukey's tests:												
Temperature		22>17>12	22>17>12	12=17>22	-	-	-	-	-	-	-	-
Origin		-	-	-	TAS>NSW	NSW>TAS	NSW>TAS	-	-	-	NSW>TAS	-
T x O		-	-	-	-	-	-	-	-	-	-	12>17>22 for NSW only

Table 2.1 (cont.)

B		Photosynthetic characteristics			Pigments			Tissue chemistry				Growth		Nucleic acids
Factor	*df*	$rETR_{max}$	E_k	F_v/F_m	Chl *a*	Chl *c*	Fucox	% C	% N	C:N	$\delta^{13}C$	Δ blade area	RGR	RNA:DNA
Temperature (T)	2	**38.5*** **	**67.9*** **	**49.9*** **	**3.58***	0.96	**10.1*** **	1.95	1.41	**4.51***	2.39	**60.6*** **	**112*** **	**15.1*** **
Nitrate (N)	2	1.47	1.21	2.04	1.94	2.71	1.55	0.31	2.16	1.87	1.81	2.38	3.20	0.38
Origin (O)	1	**11.3** **	**10.0** **	**7.30***	**53.3*** **	0.06	3.90	1.34	**14.3*** **	**27.6*** **	**165*** **	1.39	0.66	**13.2*** **
T x N	4	0.34	0.60	0.43	0.50	1.01	0.58	2.13	1.23	**2.71***	1.81	0.38	0.39	1.30
T x O	2	**9.41*** **	**3.46***	**5.94** **	0.50	0.03	0.29	0.20	0.99	1.02	0.43	0.72	2.66	**43.4*** **
N x O	2	0.14	0.04	0.70	0.67	1.44	0.58	0.18	0.16	0.64	0.08	0.42	0.78	0.27
T x N x O	4	0.18	0.18	0.13	0.08	0.65	0.13	0.64	0.32	0.18	0.31	0.06	0.100	0.55
Tukey's tests:														
Temperature		22=17>12	22=17>12	12=17>22	12=17>22	-	12=17>22	-	-	12=22>17	-	17>12>22	17>12>22	-
Origin		NSW>TAS	NSW>TAS	NSW>TAS	TAS>NSW	-	-	-	TAS>NSW	NSW>TAS	NSW>TAS	-	-	-
T x N		-	-	-	-	-	-	-	-	12>17=22 at 3 µM only	-	-	-	-
T x O		NSW > TAS at 22 °C only			-	-	-	-	-	-	-	-	-	17>22>12 (NSW); 12>17>22 (TAS)

2.4.3 Changes in survivorship, growth rate, nucleic acids and tissue chemistry

After 28 days, survivorship, growth, and RNA:DNA ratios of seaweeds all varied significantly with temperature. Survival was high (100%) at 12 and 17 °C, but reduced at 22 °C, with 4 and 5 of the 9 individuals from NSW and Tasmania (respectively) assessed (and sacrificed) early due to onset of extensive tissue necrosis. Growth of *Phyllospora* was greatest at 17 °C, followed by 12 °C, and a net loss of tissue via necrosis occurred in all individuals at 22 °C (Table 2.1B; Fig. 2.5A, B; Appendix B). Growth (absolute, or relative to initial thallus size) did not differ between NSW and Tasmanian *Phllospora* (Table 1B; Fig. 2.5A, B). Neither growth nor survival was affected by nitrate treatment (Table 2.1B), and growth at the level of individual thalli was unrelated to RNA:DNA ratios (Fig. 2.5). The response of RNA:DNA ratios to temperature depended on seaweed origin (significant Temperature x Origin interaction), with Tasmanian thalli demonstrating declining RNA:DNA ratios with increasing temperature while NSW thalli had high RNA:DNA ratios at 17 °C and 22 °C, and very low RNA:DNA (< 5) at 12 °C. Change in RNA:DNA ratios was initially driven predominantly by RNA content of tissues (Fig. 2.2B, C), but after 28 days high variability in DNA concentration (including up to a tenfold increase in absolute concentrations of DNA in Tasmanian and NSW seaweeds at 22 and 12 °C, respectively) resulted in low RNA:DNA ratios being driven largely by high DNA concentration, rather than low RNA concentration (Fig. 2.2E, F).

Phyllospora from both origins showed increases in the percentage of tissue C and N over the course of the experiment, however, Tasmanian *Phyllospora* accumulated relatively more N resulting in significantly greater % N (and thus lower C:N ratios) than NSW thalli (Table 2.1B; Fig. 2.1). The effect of nitrate levels on C:N values changed with temperature (significant Temperature x Nitrate interaction), with a slight decline in C:N with increasing levels of nitrate occurring at 22 °C. All seaweeds experienced a reduction in $\delta^{13}C$ values, but NSW thalli maintained a greater utilisation of HCO_3^- (less negative $\delta^{13}C$) relative to Tasmanian individuals (Table 2.1B; Fig. 2.1).

2.4.4 Changes in pigments and photosynthetic characteristics

Photosynthetic characteristics of seaweeds at 12 and 17 °C changed very little over the duration of the treatments (i.e. following the initial acclimation response to temperature), and

individuals from both origins exhibited similar treatment responses at these temperatures (Table 2.1B; Figs 2.3 & 2.6). However, photophysiology of Tasmanian thalli was significantly impaired at 22 °C relative to NSW individuals (Table 1B; Fig. 3). Strong evidence of stress manifested as a reduction in photosynthetic efficiency (F_v/F_m) in all seaweeds grown at high temperatures, although as with effects on $rETR_{max}$ and E_k, this was particularly severe in Tasmanian individuals. None of the photosynthetic parameters measured were sensitive to nitrate levels.

Over the course of the experiment, pigment stoichiometry shifted towards increased concentrations of fucoxanthin and decreased concentration of chlorophyll *c*, while chlorophyll *a* remained relatively consistent (Fig. 2.4). NSW seaweeds initially contained lower concentrations of accessory pigments (chlorophyll *c*, fucoxanthin), but demonstrated a proportionately greater increase in both pigments (relative to Tasmanian seaweeds) so that the final concentrations of chlorophyll *c* and fucoxanthin were indistinguishable in NSW and Tasmanian thalli (Table 2.1; Fig. 2.4). Production of both chlorophyll *a* and fucoxanthin was negatively affected at 22 °C, while nitrate treatments had no effect on pigment levels (Fig. 2.4).

2.4.5 Multivariate analysis

Multivariate ANOVA (MANOVA) on all response variables collectively revealed similar patterns of significance as many of the univariate ANOVA; namely, a strong interaction of temperature and algal origin ($F_{28,30} = 4.01$, $p = 0.0002$). Nitrates treatments had no effect on the collective multivariate physiological response ($F_{28,30} = 1.36$, $p > 0.2$).

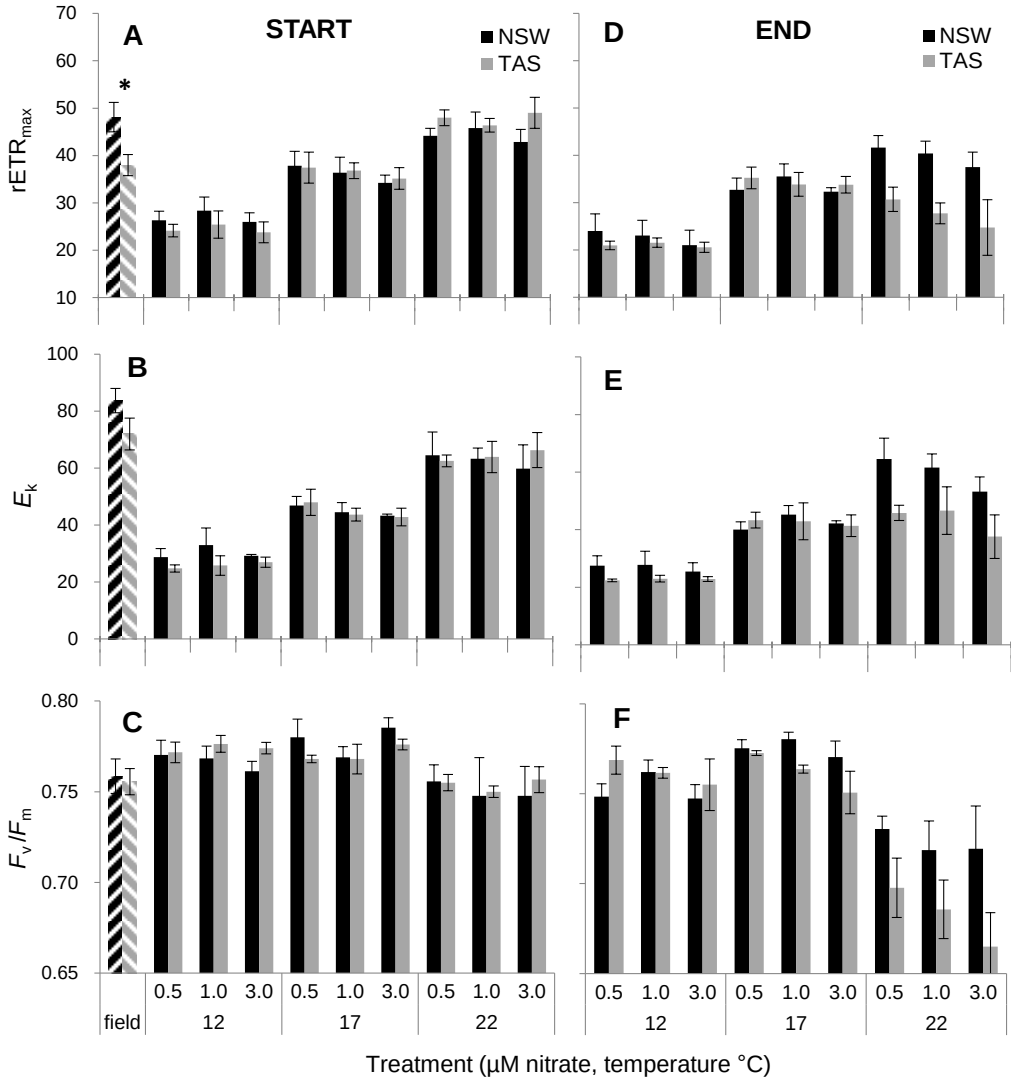


Figure 2.3 Changes in the photosynthetic characteristics of juvenile *Phyllospora comosa* originating from NSW (black bars) and Tasmania (grey bars) grown under factorial combinations of temperature and nitrate concentration. Plots show **(A, D)** maximum relative electron transport rate ($rETR_{max}$); **(B, E)** relative saturating light intensity (E_k); and **(C, F)** maximum quantum yield (F_v/F_m) derived from dark-acclimated RLCs measured by PAM fluorometry. Parameters are shown from the **(A-C)** start (diagonal stripes show *in situ* field samples, with asterisks indicating significance at $\alpha = 0.05$ in student's t-tests); and **(D-F)** end of a 28 day laboratory growth experiment. All seaweeds were acclimated to 'start' conditions for 6 days before initial sampling took place. Data from thalli that were sacrificed prematurely due to necrosis (maximum 5 days early, at 22 °C only) have been included with other 'terminal' measurements. Bars indicate mean values ($n = 3$) ± SE.

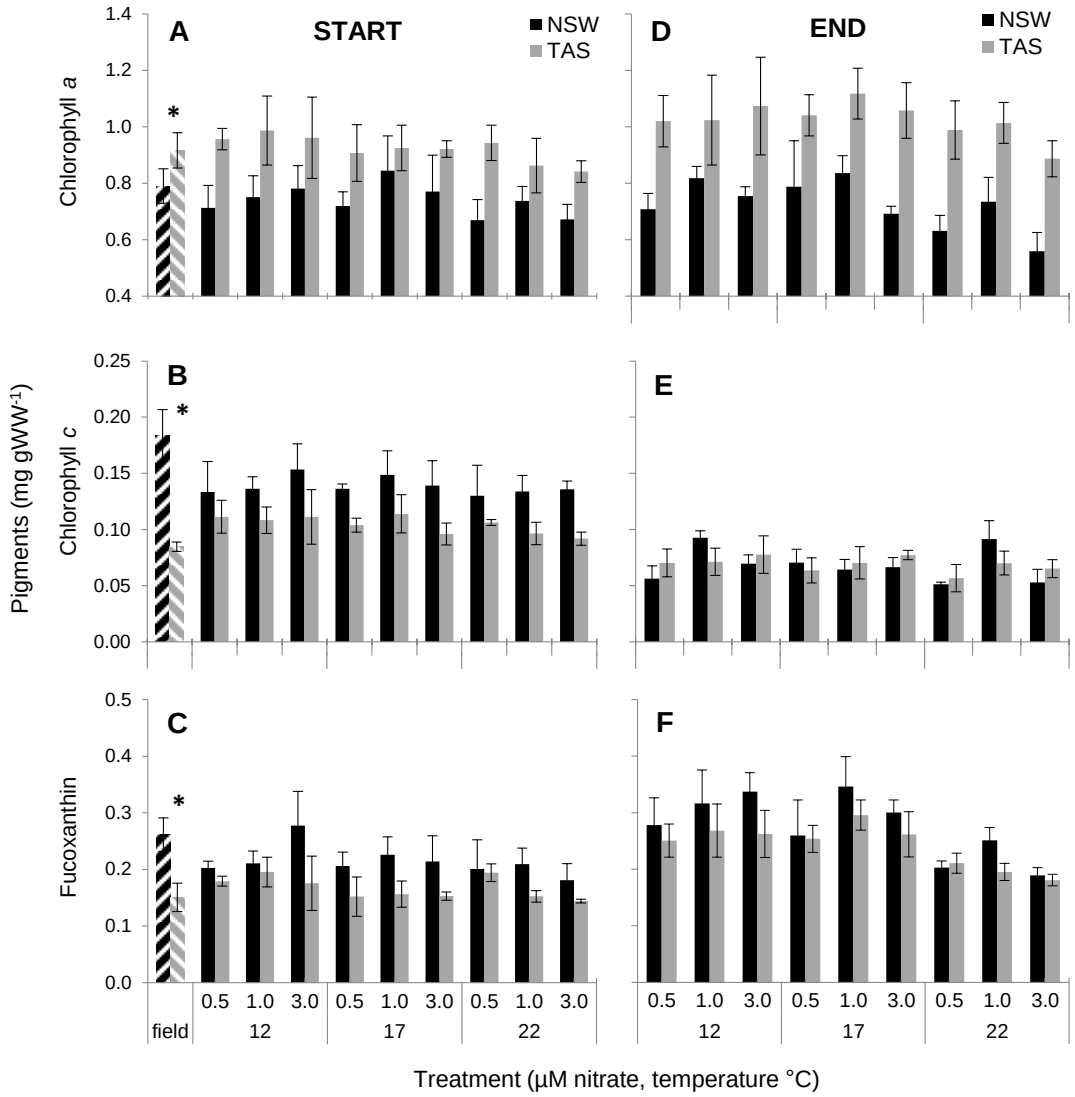


Figure 2.4 Changes in the concentration of photosynthetic pigments in juvenile *Phyllospora comosa* originating from NSW (black bars) and Tasmania (grey bars) grown under factorial combinations of temperature and nitrate concentration. Plots show **(A, D)** chlorophyll *a*; **(B, E)** chlorophyll *c;* and **(C, F**) fucoxanthin. Concentrations are shown from the **(A-C)** start (diagonal stripes show *in situ* field samples, with asterisks indicating student's t-test significance); and **(D-F)** end of a 28 day laboratory growth experiment. All seaweeds were acclimated to 'start' conditions for 6 days before initial sampling took place. Data from thalli that were sacrificed prematurely due to necrosis (maximum 5 days early, at 22 °C only) have been included with other 'terminal' measurements. Bars indicate mean values (n = 3) ± SE.

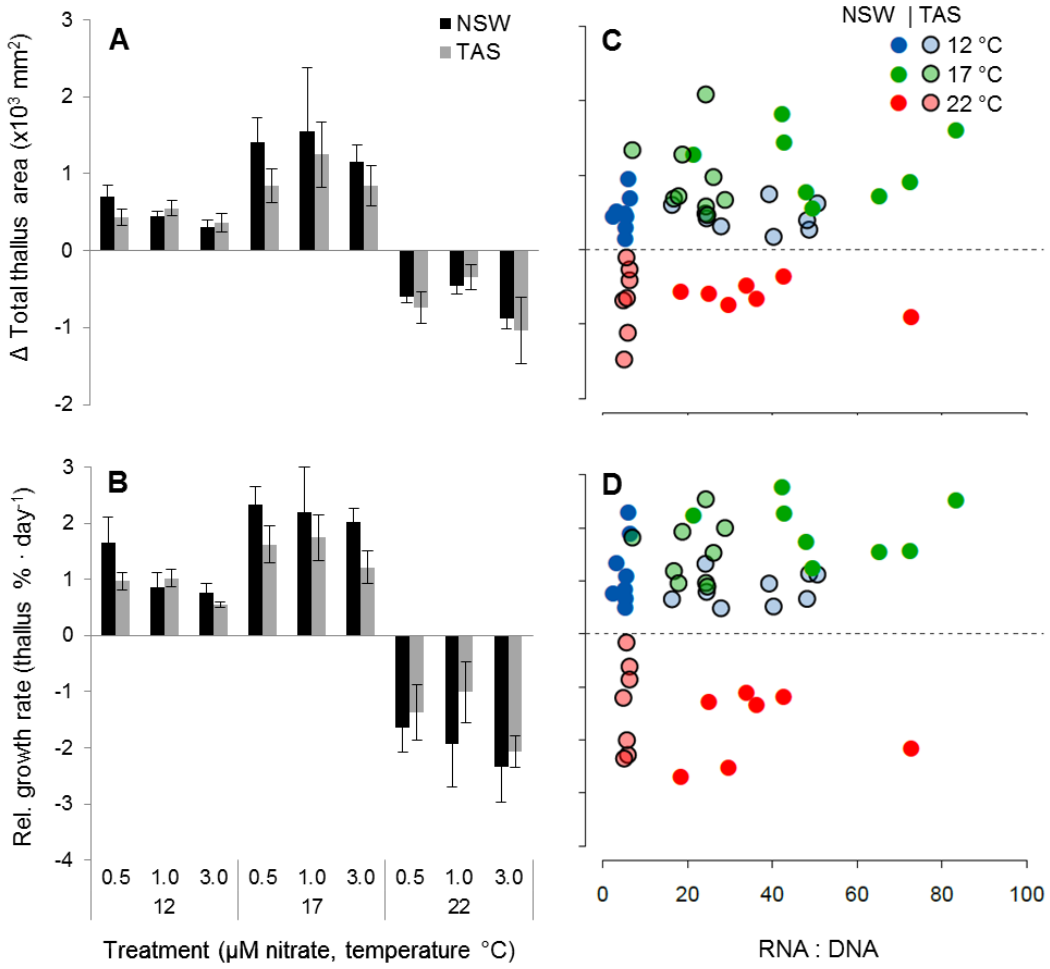


Figure 2.5 Growth and RNA:DNA ratios of juvenile *Phyllospora comosa* originating from NSW (black bars, solid borderless points) and Tasmania (grey bars, translucent bordered points) grown under factorial combinations of temperature and nitrate concentration over 28 days showing the temperature regulation of growth and RNA:DNA ratio. Plots display growth expressed as **(A, C)** change in thallus area; and **(B, D)** relative growth rate (percent per day); as means ± SE (n = 3) across factorial combinations of temperature and nitrate concentrations (bar plots), and of individual thalli as a function of RNA:DNA ratios (scatter plots). Colours in scatter plots **(C, D)** indicate temperature treatments. Where thalli were terminated prematurely due to necrosis (maximum 5 days early, at 22 °C only), growth rates were corrected for shortened experimental duration.

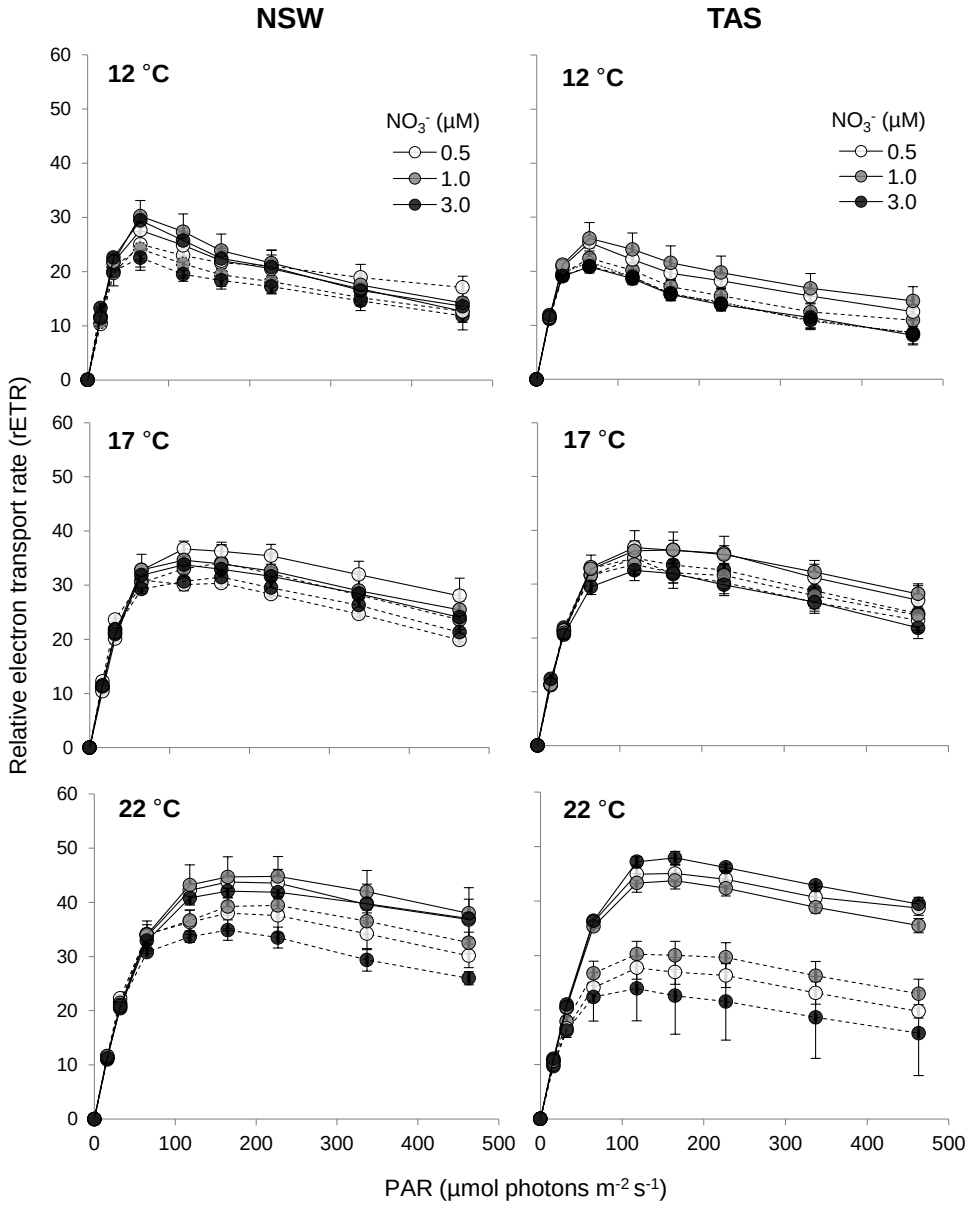


Figure 2.6 Rapid Light Curves (RLCs) derived from PAM fluorometry of dark-acclimated juvenile *Phyllospora comosa* originating from NSW and Tasmania grown under factorial combinations of temperature and nitrate concentrations. Curves are presented for each combination of temperature, nitrate and origin. Solid lines (——) indicate sampling at the start of the experiment following a 6 day acclimation period, dashed lines (- - -) indicate sampling following 28 days of growth under treatment conditions. Data from thalli that were sacrificed prematurely due to necrosis (maximum 5 days early, at 22 °C only) have been included with other 'terminal' measurements. Values expressed as means (n = 3) ± SE.

2.5 Discussion

2.5.1 Inherent physiological differences between seaweeds at NSW and Tasmanian sites

In situ sampling revealed a number of physiological differences between *Phyllospora* derived from the north and south of its range. Tasmanian seaweeds had greater chlorophyll *a* content, depleted $\delta^{13}C$ values, and a lower content of accessory pigments (relative to NSW individuals); while other ecophysiological indicators (RNA:DNA ratios, photosynthetic efficiency) were similar in seaweeds from both origins. The fraction of the heavy carbon isotope $\delta^{13}C$ is a sensitive indicator to the source of inorganic carbon used by macroalgae for photosynthesis, with algae having $\delta^{13}C$ values below -29‰ restricted to using diffusive CO_2 as a carbon source, while those that also utilise HCO_3^- via active carbon concentrating mechanisms typically have values > -25‰ (Raven et al. 2002b). The more depleted $\delta^{13}C$ values in Tasmanian thalli indicate favouring of diffusive CO_2 entry rather than the energetically more expensive process of HCO_3^- uptake and may reflect the greater ambient concentration of CO_2 (due to increased solubility) in cooler Tasmanian waters (Raven et al. 2002a). Moreover, while the surface irradiance was similar in NSW and Tasmania at the time of collection (February 2012 daily mean 5.3 and 5.1 KWh m^{-2} for NSW and Tasmania; Bureau of Meteorology 2013), for much of the year the light environment experienced by *Phyllospora* recruits in Tasmania is severely reduced due to low insolation and extensive shading by dense canopies formed by adults (E. Flukes *pers. obs.*). The lower $\delta^{13}C$ values and high chlorophyll *a* content of Tasmanian *Phyllospora* clearly demonstrates physiological adaptation to a reduced temperature and light environment, as has been widely reported in other temperate macroalgae (e.g. Altamirano et al. 2004, Fairhead & Cheshire 2004b, Miller et al. 2006).

2.5.2 Variability in physiological response times (regulation *vs.* acclimation)

The timescale over which physiological changes occurred in *Phyllospora* depended on the nature of the measurement, indicating some processes are under rapid temperature- or light-driven control via adjustment of catalytic efficiency (regulation), while others represent more gradual changes requiring net molecular synthesis or breakdown (acclimation) or are genetically fixed and thus will only change through adaptation (see Raven & Geider 2003). Photosynthetic characteristics changed rapidly (days or less) in response to water

temperature, as PAM fluorometry detects processes regulated by the activity of Rubisco and other Calvin cycle enzymes which are strongly temperature-dependent (Henley 1993, Raven & Geider 2003). The effect of temperature on RNA:DNA ratios of NSW seaweeds (which initially indicated a reduction in protein synthesis with increasing temperature, i.e. decreasing [RNA] and increasing RNA:DNA ratios with increasing temperature) changed following 28 days of treatment, with low values of RNA:DNA corresponding with low temperatures. This suggests that RNA:DNA ratios in *Phyllospora* (driven predominantly by changes in DNA concentration) are relatively labile and can change in days or less in response to temperature, but that the concentrations of both RNA and DNA may continue to change through time as seaweeds acclimate to new conditions. In contrast, pigments and chemical composition of tissues were largely unaffected by short-term exposure to temperature treatments. Interestingly, initial latitudinal differences in chlorophyll *a* and $\delta^{13}C$ did not converge over the 28 days of treatment despite seaweeds sharing a common light (and temperature and nutrient) environment. Experiments with *Ulva* have shown that carbon uptake mechanisms ($\delta^{13}C$) respond to altered irradiance in <10 days (Cornielsen et al. 2007), and coralline algae can modify their pigment composition over a matter of hours (Algarra & Niell 1990). Carbon acquisition and pigment concentrations in *Phyllospora* may therefore be less labile than has been reported for other algae. Alternatively, these characteristics may be genetically fixed in populations as has been observed with chlorophyll composition of seagrass (Durako et al. 2003).

2.5.3 Decoupling of RNA:DNA ratios from growth processes

The primary function of RNA is in protein synthesis, while DNA is the carrier of genetic information and can be used as an index of cell number or biomass (Holm-Hansen 1969, Dortch et al. 1983). The RNA:DNA ratio is thus an index of the quantity of protein synthesis machinery per cell and is a measure of an organism's 'growth potential' (Buckley 1984). However, despite the strong link between RNA:DNA ratios and growth that has been widely documented in zooplankton (e.g. Sutcliffe 1965, Sutcliffe 1969, Dortch et al. 1983, Acharya et al. 2004) and higher plants (e.g. Matzek & Vitousek 2009, Reef et al. 2010b), we found no link between RNA:DNA and growth of *Phyllospora* from either origin. This decoupling supports the observations of Reef & Lovelock (2012) who currently provide the only other study (to our knowledge) to investigate the applicability of the GRH to macroalgae. Mean RNA:DNA ratios of NSW seaweeds roughly followed patterns of mean net growth (i.e.

highest at 17 °C); however, there was no correlation between RNA:DNA ratios and growth at the level of individual thalli. Although our growth measurements incorporated losses to necrosis, this does not adequately explain the decoupling of growth potential (estimated from RNA:DNA ratios) from observed growth, as necrosis occurred exclusively at 22 °C. A lag between protein synthesis and expression of growth is also possible, but is unlikely given that seaweeds were collected in late summer when metabolic activity (expressed as growth) is generally high (Sanderson 1990, Fairhead & Cheshire 2004b, Ferrari et al. 2012). As growth is the end product of multiple simultaneously operating biochemical processes (Hasanuzzaman et al. 2013), low growth may not necessarily correspond with reduced metabolic activity. Rather, it may indicate allocation of energy towards other processes such as cellular repair (Kirst & Wiencke 1995), 'futile cycling' associated with protective metabolic processes (e.g. Britto et al. 2001), or synthesis of non-growth-related compounds such as heat shock proteins (Vayda & Yuan 1994) or antioxidant defences (Davison & Davidson 1987, Sweetlove et al. 2002, Hasanuzzaman et al. 2013).

2.5.4 Temperature regulation of growth and RNA:DNA ratios

Net growth measured as change in thallus area or biomass is frequently used as an integrated indicator of the physiological status of algae. *Phyllospora* growth increased with temperature over the range 12-17 °C, as has been demonstrated with other fucoid species (Steen & Rueness 2004). Interestingly, NSW seaweeds at 12 °C grew at similar rates to Tasmanian individuals, despite this being 3-4 °C cooler than the normal winter minima experienced by northern populations. This suggests phenotypic plasticity enabling normal growth to occur (at least over a short period) at temperatures below the normal range experienced by a population. The converse did not apply for seaweeds grown at 22 °C, where net growth (irrespective of origin) was negative due to tissue necrosis, which significantly outweighed meristematic growth. Cell death in Tasmanian *Phyllospora* at this temperature is not surprising given that it is 3-4 °C warmer than the normal summer maxima. However, NSW seaweeds regularly experience summer water temperatures in excess of 22 °C, suggesting either that small recruits are often physiologically stressed in NSW over summer, and/or that necrosis may have been a laboratory artefact due to proliferation of microalgal or bacterial contamination (Steffensen 1976) facilitated by low-flow conditions. While all seawater and air used in aeration was filtered, airborne contamination of cultures cannot be discounted, and slight water clouding was visible in 22 °C treatments despite regular media changes. Whether

microalgae and/or bacteria contributed to necrosis at high temperature (Case et al. 2010), or thermal stress caused necrosis directly cannot be determined. Regardless, these results suggest that temperatures of 22 °C and greater pose an increased stress to *Phyllospora*, and that absolute thermal tolerance may be reduced in the presence of multiple stressors characteristic of laboratory experiments (e.g. artificial cycles of irradiance, altered light quality, low flow conditions).

We identified that a precondition of high RNA:DNA ratios in *Phyllospora* was a high photosynthetic efficiency (F_v/F_m), suggesting that macroalgal photosystems must be healthy and functional to permit synthesis of RNA at high concentrations (Fig. 2.7). In contrast, Hauri et al. (2010) observed a negative relationship between F_v/F_m and RNA:DNA ratios in corals, and suggested that increasing photosynthetic stress (low F_v/F_m) resulted in elevated synthesis of stress proteins (high RNA:DNA). The discrepancies between these results may indicate fundamental differences in the role of RNA synthesis in corals and macroalgae. In general, RNA:DNA ratios of *Phyllospora* were negatively correlated with temperature (but decoupled from growth), which likely reflects temperature-driven variation in the synthesis of non-growth related proteins. For example, the concentration of Ribulose 1,5-bisphosphate Carboxylase (RuBPCase) – which catalyses the reduction of CO_2 during the Calvin cycle and comprises 20-40% of total proteins present in photosynthetic organisms (Rivkin 1990, Hurd et al. 2014) – required to maintain the same rate of C-fixation is reduced at higher temperatures. This is due to an increase in the specific activity of RuBPCase molecules with temperature (Jordan & Ogren 1984, Duke et al. 1986) due to more successful collisions of CO_2 molecules with RuBPCase active sites (Raven & Geider 1988). Decreased demand for RuBPCase or other proteins with increasing temperature may explain the observed negative relationship between temperature and RNA:DNA ratios in Tasmanian *Phyllospora*.

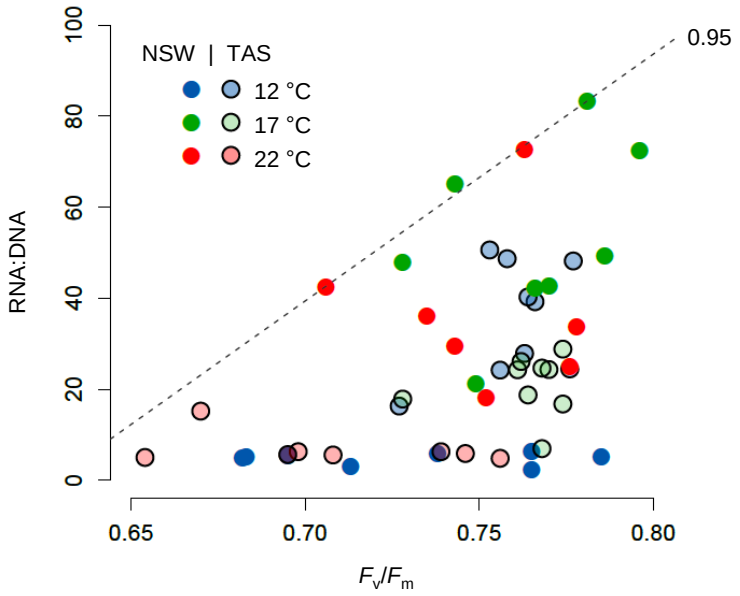


Figure 2.7 RNA:DNA ratios *vs.* photosynthetic efficiency (F_v/F_m) of juvenile *Phyllospora comosa* originating from NSW (solid borderless points) and Tasmania (translucent bordered points) following 28 days of growth under factorial combinations of temperature (12, 17, 22 °C) and nutrients (0.5, 1.0, 3.0 µM NO_3^-). Colours indicate temperature treatments. The 95th quantile regression is given by the equation $y = 542.4x - 340.4$

The seemingly anomalously low ratios of RNA:DNA in NSW *Phyllospora* at 12 °C (and, to a lesser extent, Tasmanian thalli at 22 °C) were driven predominantly by high DNA rather than low RNA content. Tissue concentrations of DNA are generally regulated by cell size and are thought to be largely insensitive to environmental changes (Dortch et al. 1983), thus variation in DNA content within individuals has been poorly studied. We cannot determine whether observed variability in DNA content was driven by changes in nuclear or plasmid-associated (chloroplast, mitochondrial) DNA, and whether changes occurred at the cellular level (due to altered size and/or density of plasmids) or at the tissue level (through variation in cell size and density). However, we suggest the spikes in DNA content of warm-adapted (NSW) seaweeds at low temperature (12 °C), and in cool-adapted (Tasmanian) seaweeds at high temperature (22 °C), may be the result of physiologically distinct processes. For example, low temperatures are known to reduce cell division times in some higher plants, resulting in smaller cell size and increased cell packing (and nuclear DNA) density (Bertin 2005).

Tasmanian seaweeds would ostensibly be unaffected in this manner by low temperatures because they are adapted to cool conditions, but reduced cell size may be responsible for increased DNA content in NSW individuals. In contrast, high DNA content of Tasmanian seaweeds at 22 °C may reflect increased mitochondrial replication in response to damage resulting from thermally-driven oxidative stress (Møller 2001, Sweetlove et al. 2002, Hasanuzzaman et al. 2013). An alternative explanation is that C-limitation may occur at 22 °C in Tasmanian seaweeds due to temperature-regulated increases in photosynthetic activity under a relatively low (fixed) light environment (Lapointe et al. 1984, Falk et al. 1996), resulting in light limitation which can stunt cell growth and increase cell packing density (Claustre & Gostan 1987). It appears clear that RNA:DNA ratios in macroalgae reflect the simultaneous operation of a number of physiological and metabolic processes, and caution must be exercised when interpreting this ratio as it represents the net balance of multiple processes affecting the absolute concentrations of both RNA and DNA.

2.5.5 Temperature regulation of photophysiology

Reduced concentrations of chlorophyll *a* and fucoxanthin at 22 °C suggests a possible loss in functionality of pigment production at high temperatures (Jokiel & Coles 1990, Case et al. 2010), presumably as a result of thermal stress (Tort & Turkyilmaz 2004, Abdul Qados 2011). However, at the two lower growth temperatures, pigment content increased over the period *t*0 - *t*28 in a consistent manner across all treatments, indicating a response to laboratory conditions (most likely light) rather than temperature- (Henley & Dunton 1995) or nutrient-driven (Falkowski & Raven 1997) effects. Declines in chlorophyll *c* were associated with large increases in fucoxanthin, which may indicate a response to the spectral quality, rather than quantity, of artificial light. Importantly, convergence in accessory pigments (chlorophyll *c*, fucoxanthin) between NSW and Tasmanian seaweeds after 28 days subject to the same treatment suggests a shared photophysiological response to environmental change and indicates that manipulation of pigment stoichiometry in *Phyllospora* is a plastic process. In contrast, chlorophyll *a* remained elevated in Tasmanian thalli suggesting an inherently greater (i.e. 'fixed') light harvesting ability in high latitude *Phyllospora* as an adaptation to growth in a relatively low light environment.

In general, a clear and rapid 'scaling' of photosynthetic characteristics occurred with increasing temperature across all seaweeds, which we interpret to indicate increased rates of molecular diffusion and enzymatic catalysis of Calvin cycle reactions (Henley 1993). However, we also observed declines in photosynthetic characteristics over time in Tasmanian *Phyllospora* grown at high temperatures. Decreased photosynthetic capacity and efficiency, as observed in these thalli, is usually attributed to a reduction in the number of photosynthetic units (PSUs) or reaction centres (Falkowski Raven 1997), and is typically associated with a concomitant reduction in chlorophyll *a* (see Fairhead & Cheshire 2004b). While we observed a slight decline in chlorophyll *a* accompanying the reduction in photosynthetic capacity and efficiency, the magnitude of pigment reduction relative to the large decline in photosynthetic performance suggests an additional loss of functionality of PSII. Declines in photosynthetic efficiency (F_v/F_m) – commonly interpreted as 'stress' – were highly significant, but relatively modest (~5-11% in NSW and Tasmanian thalli, respectively) compared with those reported for seaweed elsewhere (e.g. Karsten et al. 2001, Baumann et al. 2009, Oláh et al. 2010). With the exception of *Macrocystis* spp., most brown macroalgae are not capable of actively translocating cellular substances throughout growing tissue (Jackson 1982), so relatively healthy and functional sections of tissue may occur adjacent to necrotic blade margins. Consequently, we suggest that while F_v/F_m may reflect health of photosystems at the location on an individual where it is measured, it is unlikely to be a particularly informative measure of organism-scale physiology. The ultimate outcome for all seaweeds at 22 °C was the same (essentially 100% mortality). However, NSW seaweeds maintained consistently higher photosynthetic characteristics at 22 °C despite widespread necrosis, suggesting a reduced upper thermal tolerance of higher latitude *Phyllospora* as has been demonstrated for other fucoid species (S. Bennett *pers. comm.*). In the context of predicted climate change, there are likely to be disproportionately severe effects of increasing water temperature on *Phyllospora* in the southern portion of its range, unless there is capacity for adaptation at rates commensurate with the time scale of environmental change.

2.5.6 Lack of nitrate effects, and nitrate uptake efficiency

We found no links between nitrate availability and growth or any of the physiological parameters measured in *Phyllospora*, despite an abundance of other studies documenting the importance of nitrates in regulating productivity, nutrient storage, pigment production, RNA synthesis, and PSII photochemistry in macroalgae (e.g. Pedersen & Borum 1996, Falkowski

& Raven 1997, Reef et al. 2012). Although C:N ratios were negatively correlated with nitrate concentrations at high temperature, the magnitude of this interactive effect was weak, and likely reflects a slight increase in metabolic demands for nutrients with increasing temperature (Staehr & Wernberg 2009). The absence of any relationship between nitrate availability and accumulation or depletion of N from algal tissues suggests either that (1) nitrate concentrations administered to seaweeds were not necessarily available for uptake in the same ratios (due to depletion of nutrients in the media by other microalgal or bacterial contaminants); and/or (2) nitrate uptake efficiency increases with decreasing availability; and/or (3) nitrate uptake becomes asymptotic at concentrations ≤ 0.5 μM.

Although nitrate levels were controlled via addition of nitrate sources rather than direct testing of media, the media was changed every 5 days and the volume relative to algal size was large so that any depletion by contaminants (microalgae, biofilms) would have been minimal. An inverse relationship between N availability and uptake efficiency has been documented in terrestrial plants (see review by Vitousek 1982), but this relies on internal transport systems which most Phaeophyta (including fucoids) lack. While enrichment of phosphorus (P) typically increases uptake in P-limited tropical systems (Lapointe 1987, Reef et al. 2012), enrichment of N as NO_3^- or NH_3 appears less reliable in triggering increasing N uptake in temperate species (Chopin & Wagey 1999, Reef et al. 2012) which are typically N-limited (Pedersen & Borum 1996, Hurd et al. 2014). Given that *Phyllospora* used in this study was collected in February following several months of nitrate limitation during summer (Chapter 4), the absence of elevated N uptake under higher nitrate availability suggests that *Phyllospora* may not be capable of 'luxury uptake' (uptake of nutrients beyond immediate metabolic demand; Dring 1982), possibly due to N uptake reaching its maximum rate at concentrations ≤ 0.5 μM, or the relatively low light environment maintained throughout the experiment.

In general, nutrient requirements are lessened under low light due to reduced carbon acquisition (Lapointe & Tenore 1981, Frederiksen & Rueness 1989, Rico & Fernández 1996). While the irradiance in a static light environment (as used in this study) cannot be compared directly with natural irradiance (which is dynamically variable over milliseconds to hours), the slight reduction observed in the rate of carbon acquisition ($\delta^{13}C$), and in saturating irradiance (E_k) at the corresponding equivalent laboratory temperature (22 °C and 17 °C for NSW and Tasmanian thalli, respectively) indicates acclimation to a low light environment.

This did not prevent seaweeds from accumulating N at the same rate across all nitrate treatments, resulting in reduced C:N ratios to, or below, the value of ~15 which has experimentally demonstrated to indicate the threshold of N deficiency in macroalgae (e.g. Hanisak 1983, Lapointe 1987, Frederiksen & Rueness 1989, Rico & Fernández 1996). Moreover, although net growth was elevated at 17 °C relative to 12 °C, we found no negative effect of temperature on tissue N (due to depletion of internal N reserves through accelerated growth), suggesting increased nutrient uptake kinetics with increasing temperature as has been previously documented for macroalgae (Gerard 1982, Duke et al. 1989, Lehvo et al. 2005). Importantly, the accumulation of N by seaweeds even at nitrate concentrations below the critical threshold required for growth in other brown macroalgae (~1 µM; Gerard 1982) indicates that *Phyllospora* is highly efficient at N utilisation, and possibly storage, within the range of normal nutrient availability. This suggests that *Phyllospora* at high latitudes may be much less susceptible to climate-driven oligotrophy than is predicted by the GRH.

2.5.7 Decoupling of growth from nutrient supply (lack of support for the GRH)

Growth observed here (RGR ~1-2% day^{-1} in non-necrotic treatments) was relatively modest compared to that reported elsewhere for laminarians and fucoids in other field studies (Sidman 1983, Dean & Jacobsen 1984) and laboratory (Neushul & Haxo 1963). However, these estimates are likely to be conservative as new growth often formed curled blades (Appendix C) which were not always picked up by our two-dimensional measurements. This growth pattern was likely a result of so-called 'elastic buckling' wherein growth rates at the margins of a blade exceeded those at the midline due to lack of hydrodynamic drag forces (Koehl et al. 2008). Regardless, growth rates were similar between seaweeds from both origins despite Tasmanian individuals accumulating more N (increase in C:N of ~23% relative to increase of ~8% in NSW thalli), suggesting a greater efficiency of nutrient use and/or uptake in *Phyllospora* from high latitudes. This is in partial support of the predictions of the Growth Rate Hypothesis (GRH; Elser et al. 2003) that photosynthetic organisms at high latitudes will use nutrients more efficiently to compensate for a growth season shortened by light availability (Kerkhoff et al. 2005, Lovelock et al. 2007). However, we saw no evidence of a corresponding increase in growth of Tasmanian thalli under 'favourable' (high nitrate) conditions, or of increased sensitivity to a decrease in nutrients as is also predicted by the GRH.

The decoupling of RNA:DNA ratios (and growth) from nutrient enrichment is not surprising given that the absolute N content of tissues was unaffected by nitrate treatments. Indeed, the GRH is only predicted to apply if growth is nutrient-limited – if growth is restricted by some other environmental or biological factor, growth (and RNA:DNA ratios) should no longer be correlated with nutrient availability (Droop 1974). Reef and Lovelock (2012) suggest the GRH may not apply in macroalgae because of their considerable ability to store nutrients, suggesting instead that RNA production may be involved with futile cycling such as photorespiration and ammonium cycling. Given the nutrient treatments we administered were at concentrations within the ranges of those naturally experienced in a largely oligotrophic system, ammonium cycling is highly unlikely and photorespiration does not adequately explain the temperature regulation of RNA synthesis previously described. Clearly, further work is required to determine the precise role of RNA in macroalgae, and the ecophysiological relevance of variable RNA:DNA ratios decoupled from growth processes.

2.5.8 Conclusions

This experiment indicates that photophysiology and biochemical composition of *Phyllospora* exhibits plasticity in response to environmental conditions, particularly temperature. We found no clear evidence for a nutrient influence on seaweed performance, or interactions between (low) nutrients and temperature as has been demonstrated for other seaweeds when exposed to multiple environmental stressors (e.g. Porter et al. 1999, Lotze & Worm 2002, Kim & Garbary 2007). Within a normal environmental range, temperature (not nitrate availability) was found to be the critical factor regulating growth of *Phyllospora*. We found insufficient evidence to support the GRH in *Phyllospora*, with no detectable link between nutrient availability and RNA synthesis, or between RNA:DNA ratios and growth. Although physiological processes in seaweeds derived from low latitudes were capable of functioning at temperatures below normal environmental conditions, widespread mortality at high temperatures made it difficult to determine whether the converse applied for high latitude individuals. In the context of predicted climate change, *Phyllospora* from the cool, southern extent of its range appears better adapted to simultaneous moderate increases in temperature and relatively severe oligotrophy than is predicted by the GRH, but appears more adversely affected by high temperature than more northern populations. Therefore, while Tasmanian *Phyllospora* may be capable of withstanding short-term increases in water temperature of several degrees such as those driven by periodic warm pulses (e.g. Wernberg et al. 2013), it

appears likely that long-term temperature rises will have severe negative impacts at the individual level, which will be reflected collectively at the population scale. Furthermore, reproductive capacity may also be reduced or interrupted before the thermal threshold for growth and survival is reached (Mohring et al. 2013a). Further work should attempt to more precisely determine the upper thermal tolerance of *Phyllospora* across its latitudinal range, including cross-transplantation experiments between cold and warm sites, to facilitate predictions for the future of this species in southeastern Australia under a changing climate.

Chapter 3 Phenotypic plasticity in habitat-forming seaweeds subject to latitudinal transplant

3.1 Abstract

The ability of a species to respond to environmental change depends on both the extent of genetic variance for life-history traits at the population scale, and on the capacity of individuals to phenotypically adjust to new conditions. Macroalgae are thought to have low genetic variance relative to terrestrial plants, and rely predominantly on phenotypic plasticity to maintain high fitness over a broad range of environmental conditions. The east coast of Australia is currently one of the most rapidly changing environments in the southern hemisphere, and is also populated by dense habitat-structuring macroalgal beds. We investigated the role of phenotypic plasticity in determining the physiological success of juvenile *Ecklonia radiata* and *Phyllospora comosa* derived from low latitude (NSW; warm) populations when transplanted into high latitude populations (Tasmania; cool). Performance was assessed using a range of ecophysiological measurements: growth, photosynthetic characteristics (via chlorophyll fluorescence), pigment content, stable isotopes ($\delta^{13}C$, $\delta^{15}N$), tissue chemistry (% C, % N, C:N ratios), and nucleic acid characteristics (absolute RNA and DNA, RNA:DNA ratios). Both species exhibited a large degree of environmentally-driven plasticity, with the physiological characteristics of low and high latitude seaweeds converging over the duration of the experiment despite the occurrence of large fluctuations in photophysiology to compensate for seasonally variable abiotic conditions. *Ecklonia radiata* and *P. comosa* transplanted from low latitude responded in a similar manner to high-latitude 'home' transplants despite that water temperatures were 3-4 °C below the normal winter minima experienced by these individuals. The high levels of phenotypic plasticity in these species demonstrates their capacity to acclimatise to a wide range of environmental conditions, which may have important implications for the success (or otherwise) of these species in the context of climate change predicted for southeastern Australia.

3.2 Introduction

Variation in environmental characteristics along latitudinal gradients is a primary driver of the distribution patterns of photosynthetic organisms throughout terrestrial (e.g. Walter 1973, Woodward 1990) and aquatic (e.g. Santelices & Marquet 1998, Wernberg et al. 2010) systems. Irradiance and temperature typically decrease with increasing latitude while summer photoperiod increases, leading to latitudinal gradients in the morphology and physiology of species (Körner et al. 1991, Li et al. 1998, Santamaría et al. 2003). For example, the optimal temperature for physiological processes such as growth, survival and reproduction of seaweeds (Serisawa et al. 2001, Mohring et al. 2014) and higher plants (Chapin III 1974) tends to decrease along increasing latitudinal gradients, while morphological adaptations to decreasing radiance along latitudinal gradients include reduction in the thickness and increased surface area of photosynthetic tissues to maximise light absorption (Reich et al. 2003, Miller et al. 2011). The occurrence of multiple phenotypes enable a species to succeed across a far wider range of environmental conditions than is possible for a single phenotype.

The expression of traits in an organism (i.e. phenotype) is determined by a combination of both genetic composition and environmental influences (Scheiner 1993). Genetically controlled characteristics may only change through adaptation (e.g. Caetano-Anolles & Gresshoff 1991, Yano et al. 2001), while environmentally influenced traits can vary within individuals (see Raven & Geider 2003). If the physiological performance of a species is predominantly under genetic control, the capacity of the population to respond to environmental change will depend on genetic variation for key adaptive traits within the population. In contrast, species with phenotypes that are strongly environmentally regulated may be capable of persisting over a broad range of conditions, but this may come at a reduced fitness cost to individuals (particularly those at the margins of their physiological tolerance; Harley & Paine 2009, Wernberg et al. 2010). Terrestrial plants often possess high levels of genetic variance for physiological and morphological traits which increases their potential to adapt to new conditions and facilitates specialisation to local conditions (e.g. Bradshaw 1984, Waser & Price 1985, Joshi et al. 2001). In contrast, macroalgae appear to have relatively low genetic variance for key life-history traits (e.g. productivity, reproductive output) compared to non-genetic (environmental) sources of variation (Jormalainen & Honkanen 2004, Wright et al. 2004). Consequently, many macroalgae appear to possess general-purpose genotypes that are capable of maintaining high fitness over a broad range of environmental conditions

through morphological (Wernberg et al. 2003, Wernberg & Thomsen 2005, Fowler-Walker et al. 2006, Stewart 2006) and/or physiological (Steneck et al. 2002, Wernberg et al. 2010) adjustments.

The relative contribution of genotype and environmental factors to the expression of a given phenotype can be assessed using transplant experiments. These frequently take the form of reciprocal transplants, which provide a useful tool for separating the effects of spatial gradients in one or more environmental factors on the regulation of a species' distributions or community interactions (Harley 2003, Crain et al. 2004, Angert & Schemske 2005, Genton et al. 2005). One-way transplants, in which differences in the performance of 'home' individuals *versus* those transplanted from a different environment are assumed to reflect the extent of local adaptation (e.g. Waser & Price 1985, Joshi et al. 2001, Santamaría et al. 2003), can also be used as an assessment of the extent of environmental influence on trait expression. Given the increasing importance of environmental change in regulating species distributions (see reviews by Walther et al. 2002, Parmesan & Yohe 2003, Harley et al. 2012) and community structure (Poulard & Blanchard 2005, Frenette-Dussault et al. 2013, Velásquez-Tibatá et al. 2013), it is not surprising that transplant experiments have begun to use spatial gradients in climate to serve as a proxy for predicted temporal changes in climate (e.g. Nadkarni & Solano 2002, Etterson 2004, Berkelmans & van Oppen 2006). However, transplant experiments in marine systems have typically focused on movement of macroalgae across depth (Stengel & Dring 1997, Hoyer et al. 2001, Karsten et al. 2001, Durako et al. 2003) or wave exposure (Blanchette 1997, Fowler-Walker et al. 2006) gradients, and little is known about the extent of physiological plasticity in seaweeds responding to manipulation of environmental conditions (via latitudinal transplant) in the field.

Measuring the extent of physiological plasticity in macroalgae requires robust ecophysiological assessments to be made under natural conditions. While *in situ* studies of whole seaweed thalli are becoming more prevalent in the ecophysiological literature (e.g. Cheshire et al. 1996, Beer et al. 2000, Durako & Kunzelman 2002, Gévaert et al. 2003, Fairhead & Cheshire 2004a, Cornielsen et al. 2007), these studies typically utilise single measurements of growth (linear extension or biomass; e.g. Wheeler & North 1980, Dawes et al. 1994, Altamirano et al. 2000), photosynthesis (Beer & Bjork 2000, Durako & Kunzelman 2002, Belshe et al. 2008, Ekelund et al. 2008) or productivity (Hanelt et al. 1997, Fairhead & Cheshire 2004a, Fairhead & Cheshire 2004b) to characterise physiological performance.

However, univariate measures can fail to detect organism-scale adaptive responses to changed environmental conditions, thus a multivariate approach provides a more robust assessment of potential physiological change. This study investigates the role of environmental conditions in determining the physiological performance of two key habitat-forming seaweeds, *Ecklonia radiata* and *Phyllospora comosa* (hereafter *Ecklonia* and *Phyllospora*), in southeastern Australia. Seaweeds were transplanted from the northern (NSW; warm) to southern (Tasmania; cool) part of their range at the end of summer (28th February 2012) and monitored for four months until winter (30th June 2012). We used a unique multivariate methodology to examine changes in a range of performance and ecophysiological indicators (photosynthetic characteristics, growth, pigment concentrations, % C and % N, C:N and RNA:DNA ratios, $\delta^{13}C$ and $\delta^{15}N$). This work aimed to (1) compare the performance of *Ecklonia* and *Phyllospora* phenotypes between northern and southern populations; (2) determine the extent of phenotypic plasticity in these species in response to changed environmental conditions (resulting from transplantation), and (3) isolate the underlying physiological mechanisms permitting acclimation to novel abiotic conditions.

3.3 Materials and Methods

3.3.1 Study species

Phyllospora comosa (Fucales) dominates the shallow high-wave energy zone on rocky reefs from Robe (South Australia) to Port Macquarie (NSW) and around Tasmania (Campbell et al. 2014), typically growing from 0 to ~5 m depth but extending below ~10 m on exposed coastline. Thalli grow to 3 m in length, with a short stipe bearing multiple primary branches. *Ecklonia radiata* (Laminariales) has a larger latitudinal range (27.5-43.5 °S) than any other habitat-forming seaweed in Australia and is typically found immediately below the shallower *Phyllospora* band down to a depth of 60+ m (Nelson et al. 2014).Thalli grow up to 2 m in length with a single stipe bearing a flattened blade and distinct lateral fronds.

3.3.2 Seaweed collection and experimental transplant

The role of phenotypic plasticity in determining the physiological success of seaweeds was tested using a one-way transplant experiment, whereby seaweeds collected in NSW (warm conditions) were transplanted to cooler conditions (Tasmania) and their performance

assessed. A control group was also established where seaweeds were collected in Tasmania, treated in the same way as NSW thalli (except that they were not transported by air), and transplanted back to the same site. Juvenile *Phyllospora* and *Ecklonia* were collected between 25th-27th February 2012 from depths of ~5 and ~10 m, respectively, on exposed rocky coast near Nelson Bay, NSW (32.791° S, 152.107° E) and in Fortescue Bay, Tasmania (43.123° S, 147.976° E). Attempts were made to collect thalli of a similar size (*Ecklonia* Stage 1 sporophytes; *sensu* Kirkman 1981); however, NSW *Ecklonia* and *Phyllospora* were significantly larger than Tasmanian individuals (Table 3.1) because small individuals were subject to heavy sea urchin grazing. Immediately following collection, thalli were transferred to aerated filtered seawater and held under low light (~10 µmol photons m^{-2} s^{-1}) and temperature (13-15 °C) for 24 hours during which time they were transported to the laboratory.

Table 3.1 Size characteristics of juvenile *Ecklonia radiata* and *Phyllospora comosa* collected from New South Wales (NSW) and Tasmania. Measurements indicate the length and width of the longest blade/lateral on each thallus. Blade length and area are expressed as means (n = 20) ± SE Significantly larger values (Student's t-test) are shown in **bold**. *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

Size characteristics	*Ecklonia radiata*		*Phyllospora comosa*	
	NSW	Tasmania	NSW	Tasmania
Size range (blade length, mm)	76-154	63-135	71-204	64-203
Mean blade length (mm)	104 ± 4.8	95 ± 4.4	**118 ± 7.1****	87 ± 7.0
Mean blade area (x10^2 mm^2)	**33.5 ± 2.2***	27.9 ± 1.9	**28.1 ± 2.2*****	17.6 ± 1.8

Once in the laboratory, thalli were attached to lengths of 6 mm diameter polypbropylene rope (while submerged in seawater) by inserting holdfasts into the weave of the rope using blunt-tipped tweezers. *Ecklonia* thalli were spaced at 100 mm intervals with 10 plants per rope (2 ropes, n = 20 for each site of origin), and *Phyllospora* were spaced at 150 mm intervals with 5 plants per rope (4 ropes, n = 20 for each site of origin). Thalli from the two regions were randomly interspersed with each other along ropes to minimise any potential effect of position on the rope. Ropes bearing seaweed thalli were deployed at Fortescue Bay, Tasmania on 28th and 29th February 2012 (~48 hours following collection). Ropes holding *Ecklonia* were aligned horizontally on the bottom between two fixed weights at a depth of 10

m within monospecific stands of adult *Ecklonia*. Because of drag and scouring by attached and drift adult *Phyllospora,* ropes holding *Phyllospora* were placed vertically at a depth of 4-5 m from a fixed weight on the substratum to a subsurface float. *In situ* irradiance measurements showed minimal variation in light between different positions on vertical *Phyllospora* ropes. Reef structure and water movement was homogenous along each depth contour and the order of ropes was randomised, so it was assumed that the effect of 'rope' (i.e. 'position') was negligible. A unique identifier was assigned to each thalli so that individuals could be identified on subsequent assessment occasions for the purposes of growth measurements.

3.3.3 Sampling schedule, environmental characterisation, and ecophysiological measurements

To provide a baseline at the time of seaweed collection, *in situ* pulse-amplitude-modulated (PAM) fluorometric measurements were taken from a randomly selected set of five individuals in each location, and tissue collected from each for analysis of pigment content, tissue chemistry, stable isotopes, and nucleic acids (see specific sub-sections below for further detail on all sampling techniques described above). To minimise positional variation in tissue characteristics, the sampled area was standardised as one-third towards the distal end of the primary lamina from the top of the stipe, and one-third along the branch/lateral. A section (> 60 mm^2) of the thalli was torn from alga using gloved hands and sealed in lightproof containers which were brought to the surface. Tissue was removed from containers at the surface but maintained under low light and rinsed with de-ionized water before being cut into three pieces for separate analyses (see below in this section for further details). All samples were kept on ice in darkness for transport immediately following collection and were refrigerated or frozen (as appropriate) within six hours of collection.

Non-destructive sampling was conducted on seaweed thalli approximately 1 hour following plant-out into the field, and again every ~4 weeks until 30th June 2012 (5 assessment in total). The size of all thalli was measured (n = 20 at the time of transplant), and PAM fluorometry was conducted on a randomly selected set of n = 10 individuals (see more below). Upon termination of the experiment, n = 10 randomly selected individuals were destructively sampled for the full suite of ecophysiological measurements. Tissue was collected from thalli *in situ* as previously described and brought to the surface. For tissue chemistry and stable

isotopes (% C, % N, $\delta^{13}C$ and $\delta^{15}N$), 10-20 mm^2 of tissue was rinsed with de-ionized water before freezing at -20 °C for mass spectrometric analysis. A similar sized piece of tissue was removed, rinsed, and stored in 2 ml of RNAlater (Ambion, Inc., Austin, TX), a nontoxic RNA stabilization buffer, for nucleic acid (RNA, DNA) measurements. These samples were refrigerated overnight at 4 °C before being transferred to -80 °C for long-term storage in accordance with the recommendations of Ambion. A third section of tissue 20-40 mm^2 in size was rinsed and stored in darkness at -20 °C for pigment analysis.

Environmental characterisation

Water temperature data and photosynthetically active irradiance (PAR) data were collected as described in Chapter 4 using a YSI 6600V2 sonde (YSI Inc., Yellow Springs, OH, USA) and Odyssey PAR logger (Dataflow Systems, New Zealand). Additional water temperature data was also collected by a nearby offshore monitoring buoy at Maria Island, Tasmania (42.597 °S, 148.233° E; Integrated Marine Observing System 2013).

Growth rates

We did not use the hole-punch method (originally developed by Mann 1972) for measuring meristematic growth because of potential issues associated with infection and impairment of photosynthetic ability in these very small thalli. Instead, we determined the length and width of blades/lamina as a proxy for thalli size. 'Length' and 'width' measurements of thalli were taken from top of the holdfast to the tip of the furthest blade (*Phyllospora*) or central lamina (*Ecklonia*), while width was determined as the maximum width of the longest blade (*Phyllospora*) or main lamina (*Ecklonia*). Where lateral branches began to form in *Ecklonia* towards the end of the experiment, width measurements of thalli were taken as the distance between tips of the longest pair of opposite laterals. An index of 'blade area' was calculated (as length x width). Relative growth rates (RGRs) per day as a function of blade area were calculated as: 100[ln(final area) – ln(initial area)]/days after Kain and Jones (1976).

Pulse amplitude modulated (PAM) fluorometry

Photosynthetic activity was estimated by variable chlorophyll *a* fluorescence using a blue LED Diving-PAM (Walz, Germany). Seaweed thalli were connected to a "dark leaf clip" (as termed by Walz) to maintain a consistent spacing of the PAM fiber optic from the surface of the tissue and permit fluorescence measurements without ambient light interference. An inbuilt software routine was used to generate rapid light curves (RLCs), where actinic light

intensity was increased in eight steps (reaching a maximum of 463 µmol photons m^{-2} s^{-1} at 650 nm) of 10 seconds in duration. RLCs were conducted on 15–20 minute dark-acclimated tissue (determined by our pilot studies to be sufficient to allow oxidation of the electron transport chain and relaxation of photoprotective mechanisms; i.e. further dark-acclimation resulted in no further increases in F_m or F_v/F_m; Appendix A). While ambient light-acclimated RLCs demonstrate the thalli's immediate light history and can be affected by recent canopy shading, water clarity, cloud cover etc., dark-acclimation 'standardises' the pre-manipulation light history and reflects the inherent physiological state of photosystems beyond the immediate light history (Ralph & Gademann 2005). All PAM fluorometry was conducted between 12:00 and 14:00 to further minimise potential variability in photosynthetic activity due to diurnal cycles of non-photochemical quenching (e.g. Belshe et al. 2007). All RLCs were conducted *in situ*, with the leaf clip moved 20-30 mm between initiations of each RLC to avoid light stress affecting subsequent fluorometric measurements.

Curves were fitted to raw rETR data to derive the saturating light intensity (E_k) and maximum electron transport rate, or photosynthetic capacity ($rETR_{max}$). The maximum quantum yield, or intrinsic potential efficiency of PSII (F_v/F_m), was also derived from dark-acclimated samples (see Falkowski & Raven 1997). The double exponential decay model of Platt et al (1980) was selected as the model fit because it adequately described both the initial linear response and the region of photoinhibition (Ralph & Gademann 2005):

$$P = P_s\left(1 - exp\left(\frac{-\alpha E_d}{P_s}\right)\right) \times exp\left(\frac{-\beta E_d}{P_s}\right)$$

where P is the photosynthetic rate (here used as rETR), α is the light harvesting efficiency as measured by the initial slope of the RLC before light saturation, E_d is the downwelling actinic irradiance of the PAM's internal halogen light, β is the negative slope of the RLC at high irradiance, and P_s is a scaling factor defined as the maximum potential rETR in the absence of any photoinhibition (if $\beta = 0$, this is equal to $rETR_{max}$). Nonlinear least-squares function using the 'R' software environment (v 3.0.0) was used to fit models, and the parameters $rETR_{max}$ (maximum relative electron transport rate) and E_k were estimated as per the equations given in Ralph and Gademann (2005). The following parameters were used in the curve-fitting routine to ensure convergence: iterations = 100; stepsize = 1/1024; tolerance =

0.00001; initial seed value for P = maximum rETR derived from raw data, α = slope of linear regression fitted to first three points of raw data (typically in the range 0.7 – 1.0).

Elemental composition of tissue

Frozen seaweed samples were thawed, blotted dry and freeze dried for 18 hours before being ground and homogenised. Approximately 5 mg of each dried, powdered sample was weighed into tin cups (Elemental Microanalysis LTD., Okehampton, UK) which were closed prior to analysis. Carbon, nitrogen, $\delta^{13}C$ and $\delta^{15}N$ content were determined using a Thermo Gas Chromatograph coupled to a Finnigan Mat Delta S isotope radio mass spectrometer operating in continuous flow mode. Results are presented in standard sigma notation:

$$\delta^{13}\text{C or } \delta^{15}\text{N } (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \qquad \text{where R} = {}^{13}\text{C}/{}^{12}\text{C or } {}^{15}\text{N}/{}^{14}\text{N}$$

Externally validated working standards were replaced every 12 samples, with Pee Dee Belemnite (VPDB) used throughout as a standard for carbon and air as a standard for nitrogen. Within-sample variability from a subset of duplicate samples (>20% of total samples run) gave the variability for *Ecklonia* and *Phyllospora* (respectively) as follows: carbon: ± 2.2 and 1.8%; nitrogen: ± 3.3 and 1.9%; C:N: ± 3.5 and 2.1%; $\delta^{13}C$: ± 1.6 and 2.0%; $\delta^{15}N$: ± 6.3 and 3.6% (mean percentage standard error of duplicate samples).

RNA and DNA determination

Absolute cellular concentrations of RNA and DNA (and RNA:DNA ratios) were used to determine the proportion of protein synthesis machinery per cell as a proxy for "growth potential" of thalli. RNA and DNA content were determined by extraction from a single tissue sample. Frozen samples stored in RNAlater were thawed, patted dry, and a 1-5 mg (wet weight) subsample was weighed and homogenised with a drill pestle in a solution containing 500 µL of urea (4 M), sodium dodecyl sulphate (SDS, 1%), trisodium citrate (1 mM), sodium chloride (0.2 M) and 5 µL of Proteinase K (A.R. Bridle *unpub. data*). To ensure complete cell lysis, digestion of RNAses by Proteinase K, and stabilisation of nucleic acids, the homogenised solution was incubated at 37 °C for 10 minutes before being placed on ice. 750 µL of ammonium acetate (7.5 M) was added to the cooled solution, vortexed and centrifuged for 5 minutes at 14,000 RCF to precipitate impurities (e.g. chlorophyll, phenolic compounds, salts, detergents in urea/SDS, etc.). The resulting supernatant, containing total nucleic acid

(tNA), was decanted into a 1.5 ml tube and precipitants discarded. 700 µL of isopropanol was added to the supernatant and tNA was pelletised by gently inverting the tube 40 times before centrifuging for 10 minutes at 14,00 RCF. The tNA pellet was washed twice in a 75% ethanol (EtOH) solution, resuspended in 200 µl molecular grade H_2O and split into two 100 µL aliquots.

A solution of 20 µL 10x DNase I reaction buffer (New England Biolabs – B0303S), 5µL DNase I (New England Biolabs - M0303L) and 80 µL molecular grade water was added to one aliquot of tNA suspension for total DNA digestion, and 5 µL of RNAse A (Sigma Aldrich - R6148) with 100 µL of molecular grade water was added to the other aliquot to digest total RNA. Both tubes were incubated at 37 °C for 20 minutes to facilitate digestion (as per supplier's recommendations) then stabilised on ice. Impurities were further precipitated by vortexing in 400 µL of a urea/SDS buffer followed by vortexing and centrifuging with 200 µL of ammonium acetate (7.5 M). Isopropanol was added to the two aliquots of supernatant and total RNA and DNA precipitated as per the previous step. RNA and DNA pellets were washed twice in 75% EtOH and resuspended into 100 µl of molecular grade water (RNA) or EB buffer (DNA). Final concentrations of RNA and DNA were quantified by fluorescence assays performed using a Quibit assay probe and fluorometer. Nucleic acid concentrations were expressed as total RNA and DNA (µg.g wet weight^{-1}) which were used to calculate RNA:DNA ratios. Within-sample variability from a subset of duplicate samples (>20% of total samples extracted) gave the variability for *Ecklonia* and *Phyllospora* (respectively) as follows: total RNA: ± 15.1 and 14.8%; total DNA: ± 17.5 and 17.0%; RNA:DNA: ± 20.1 and 14.2% (mean percentage standard error of duplicate samples).

Pigment extraction

Frozen samples collected for pigment extraction were thawed and blotted dry, then weighed to the nearest 0.01 mg. Approximately 100-150 mg (wet weight) of seaweed tissue was transferred to a 15 ml vial and extracted into 5 ml of N,N-dimethylformamid (DMF). Processing was conducted rapidly under low light to minimise sample deterioration or change in pigment content resulting from exposure to ambient light. Vials were wrapped in aluminium foil and left in total darkness for 96 hours at -20°C for extraction to take place. Following extraction, 2 ml was removed, centrifuged at 8,000 rpm for 8 minutes and frozen at -80 °C for High Performance Liquid Chromatography (HPLC) analysis.

Centrifuged samples for fucoxanthin determination were measured in 2 µl aliquots using a Waters Acquity H-series UPLC™ (Waters Corporation MA. USA) coupled to a Waters Acquity Photodiode Array Detector (PDA) with an Ethylene Bridged Hybrid (BEH) C-18 column (2.1 x 100 mm x 1.7 micron particles). The mobile phase was a gradient mixture prepared from Merck Chemicals (Merck KGaA, Darmstadt, Germany) with three eluents: 1% acetic acid, acetonitrile, and 80:20 methanol:hexane. Initial conditions were a mixture of 20% acetic acid and 80% acetonitrile for 3.5 minutes, followed by an immediate switch to 80% acetonitrile and 20% methanol:hexane which was then held for a further 5.5 minutes. Samples were left for 3 minutes and allowed to re-equilibrate. Initial calibration of the UV-Vis response at 440 nm for fucoxanthin was carried out on a fresh, accurately prepared standard solution (Sigma Aldrich, Castle Hill, Australia) made up at 1.26 µg mL^{-1} in methanol. The column was held at 35 °C with a flow rate of 0.35 mL $minute^{-1}$ throughout. Chromatograms were extracted at 440 nm from the raw data using Waters TargetLynx V4.1 software. The area beneath the fucoxanthin peak was recorded and converted to mg mL^{-1} fucoxanthin, then to mg g^{-1} wet weight.

Chlorophyll *a* and *c* content was measured from the remaining ~3 mL of DMF extracted sample using a Dynamica HALO RB-10 Spectrophotometer and processed using UV Detective software (v 1.1). Samples were spectrophotometrically measured at the wavelengths 664.5, 631, and 582 nm. Where absorbance was above 1.000, samples were diluted with DMF until readings dropped below 1.000 in accordance with the manufacturer's recommendations. DMF was used as a blank throughout.

The following equations were used to calculate pigment content (expressed as mg ml^{-1}):

$$[\text{Chlorophyll } a] = (12.7 * E_{\lambda 664.5}) / 1000 \qquad \text{(Inskeep \& Bloom 1985)}$$

$$[\text{Chlorophyll } c] = (E_{\lambda 631} - E_{\lambda 582}) - (0.297 \times E_{\lambda 664.5}) / 61.8 \qquad \text{(Seely et al. 1972)}$$

where E_x = the measured absorption coefficients at wavelength of *x* nm.

Pigment concentrations were then converted to mg g^{-1} wet weight.

3.3.4 Statistical analysis

Growth metrics (blade area and relative growth rate) were plotted against time and described by exponential fitted curves (blade area) and linear regression (relative growth rate). Data were transformed as necessary to achieve a normal distribution of residuals around the regression line (log-transformation required for all metrics), with an adjustment of (largest 'negative growth' value + 0.001) added to RGR data to correct for negative growth values. Differences between linear relationships (i.e. differences between growth of NSW and Tasmanian thalli) were tested for each species using ANCOVA, with 'time since transplant' as the covariate. Where 'time since transplant' (5 levels: monthly sampling) by 'latitude of origin' interactions were not significant ($p > 0.25$), ANCOVA was repeated without the interaction term.

For all other response variables ($rETR_{max}$, E_k, F_v/F_m, % C, % N, C:N, $\delta^{13}C$, δ^{15}, absolute RNA and DNA, RNA:DNA ratios, chlorophyll *a* and *c*, fucoxanthin), 2-way ANOVA (main factors 'time since transplant' and 'latitude of origin') was conducted on each species to determine the significance of differences in physiological responses of NSW and Tasmania seaweeds to transplantation. Although PAM fluorometric measurements were taken monthly, in the ANOVA we only compared two levels of 'time since transplant' (*in situ* 'baseline' *vs* final measurements) as we were *a priori* interested in how photosynthetic parameters four months following transplantation differed from *in situ* parameters. Metrics derived from destructive sampling (tissue chemistry, nucleic acids, pigments) also had two levels of 'time since transplant' (baseline and final). For all ANOVA, data were checked for conformity to the assumptions of homoscedasticity and normality of residuals. Where heteroscedastic, the transformations to stabilise variances were determined by the relationship between SDs and means of groups (Draper & Smith 1981). Where significance was detected by ANOVA, *a posteriori* multiple comparisons were conducted using Tukey's HSD test. The statistical package 'R' (v 3.0.0) was used for all analyses.

3.4 Results

3.4.1 Survival and growth rate

Survival of seaweeds could not be determined directly, because the absence of a particular individual from a rope may have been due to physical abrasion by drift algae wrapping around the rope, removal by grazers, or senescence. Of the 20 individuals of each species and origin initially transplanted, at least 10 were retained at the conclusion of the experiment (*Ecklonia*: n = 11, 15; *Phyllospora*: n = 10, 11; from NSW and Tasmania, respectively). Although absolute net growth (measured as absolute change in blade area index) was highly variable between thalli due to varying degrees of erosion, all seaweed groups demonstrated positive mean growth (Fig. 3.1). NSW *Ecklonia* were ~20% larger than Tasmanian *Ecklonia* at the start of transplant (see Table 3.1), so it is not surprising that overall they showed higher growth rates than Tasmanian thalli (although not when corrected for initial size, i.e. RGR). The reverse was true for *Phyllospora* with NSW and Tasmanian thalli converging in size over the four months following transplantation (Table 3.2; Fig. 3.1) despite NSW thalli being initially ~60% larger than those from Tasmania (Table 3.1). The RGR of seaweeds (both species) was seasonally consistent (~0.5-1.5% increase in thallus area per day depending on species and site of origin), however Tasmanian *Phyllospora* maintained a greater RGR than NSW thalli, while *Ecklonia* from both sites of origin maintained similar RGRs (Table 3.2; Fig. 3.1).

Table 3.2 Results of two-way ANOVA (photosynthetic characteristics, pigments, tissue chemistry, nucleic acid ratios) and ANCOVA (growth) testing for physiological changes in *Ecklonia radiata* and *Phyllospora comosa* originating from two sites (NSW, Tasmania) before (*in situ*) and four months following transplantation NSW→TAS and TAS→TAS. For growth metrics (blade area, RGR) the factor 'time' has 5 levels representing monthly sampling (t_0 sampled on the day of transplant) and was used as a covariate in the analysis. Abbreviations: chlorophyll *a* (chl *a*), chlorophyll *c* (chl *c*), fucoxanthin (fucox), relative growth rate (RGR). Only degrees of freedom (*df*), *F*-values and significance levels are shown. Significant tests are shown in bold. *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$. [#]interaction term removed from model as $p > 0.25$.

Factor	*df*	Photosynthetic characteristics			Pigments			Tissue chemistry					Nucleic acids			*df*	Growth	
		F_v/F_m	$rETR_{max}$	E_k	Chl *a*	Chl *c*	Fucox	% C	% N	C:N	$\delta^{13}C$	$\delta^{15}N$	RNA	DNA	RNA:DNA		blade area	RGR
ECKLONIA																		
Origin	1	**14.5****	3.85	1.15	0.05	0.17	**10.9****	2.74	**10.6****	**6.97***	0.39	0.05	1.27	1.79	2.01	1	**17.7*****	3.47
Time	1	**17.2*****	**44.3*****	**10.7****	**6.79***	**19.9*****	**30.3*****	4.04	0.70	0.01	0.02	**48.2*****	0.48	3.34	2.62	4	**36.1*****	1.95
O x T	1	**6.00***	**4.43***	0.11	0.69	0.73	0.28	0.11	**5.80***	**6.98***	3.99	0.01	0.22	0.77	0.72	4	**5.51***	-[#]
residuals	27															134		
PHYLLOSPORA																		
Origin	1	0.00	3.90	1.64	**7.52***	0.59	3.51	1.12	0.80	0.06	**6.49***	0.43	2.40	0.01	1.88	1	**12.0*****	**7.35****
Time	1	**5.14***	**27.6*****	**63.2*****	**33.3*****	0.11	**166*****	**18.6*****	2.05	**11.8****	1.72	**24.0*****	3.10	**33.8****	**40.5*****	4	**181*****	1.32
O x T	1	0.09	1.62	1.11	0.05	**13.9****	1.70	1.82	3.35	**4.55***	**23.6*****	0.29	0.09	1.35	0.00	4	**8.45****	-[#]
residuals	27															108		

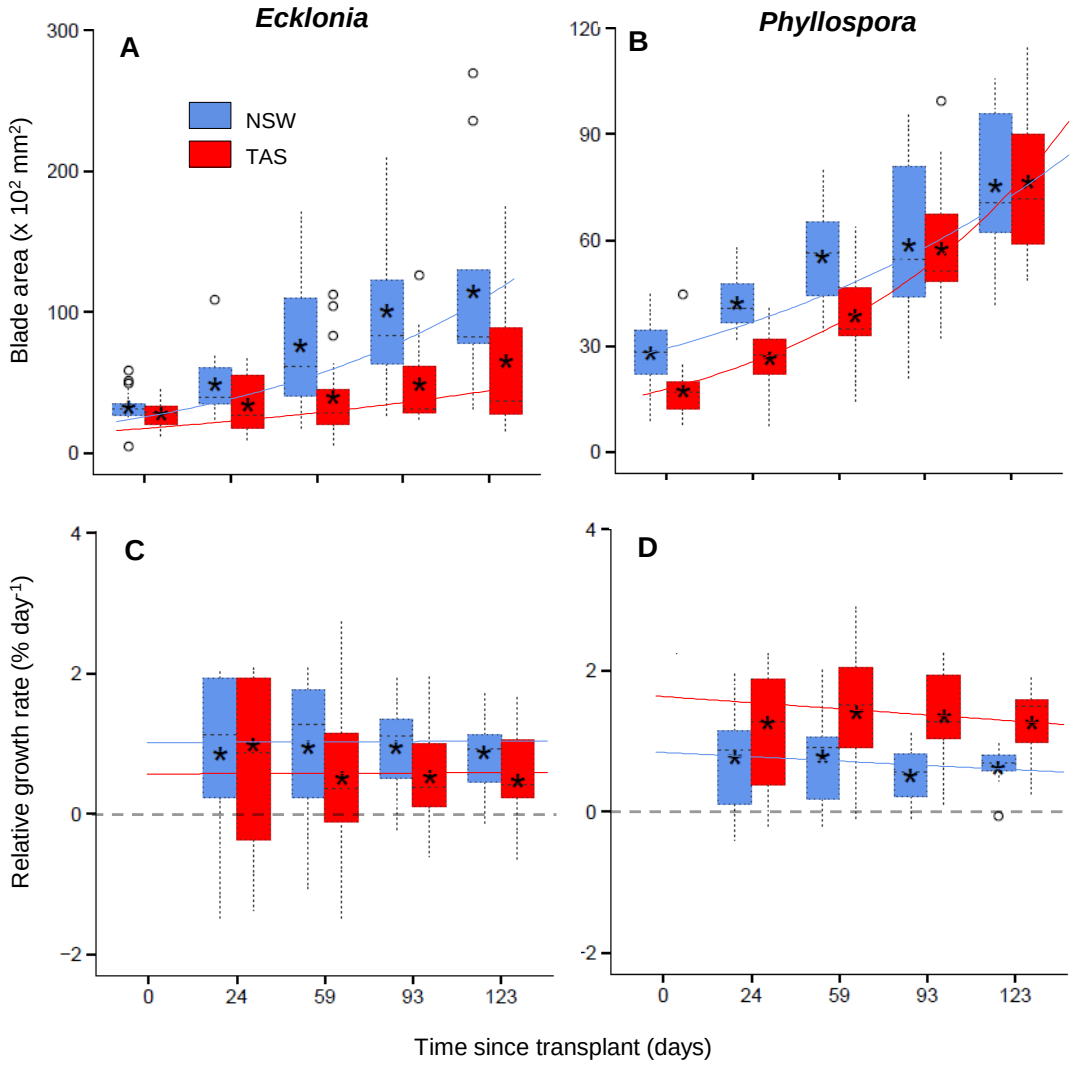


Figure 3.1 Growth of juvenile *Ecklonia radiata* and *Phyllospora comosa* originating from NSW and Tasmania following transplantation to Tasmania: **(A, B)** blade area; and **(C, D)** relative growth rate calculated as 100[ln(final area) – ln(initial area)]/(days since transplant). Measurements were taken approximately every 28 days commencing in February 2012 when transplantation occurred. Boxplots show 25-75th percentiles with median line, with outliers indicated by (○) and means shown by (★). Coloured lines indicate regressions fitted to means. **(A)** *Ecklonia:* NSW, $y = \exp(0.0102x + 3.46)$, $R^2 = 0.381$; TAS, $y = \exp(0.00566x + 3.24)$, $R^2 = 0.094$; **(B)** *Phyllospora*: NSW, $y = \exp(0.00785x + 3.35)$, $R^2 = 0.533$; TAS, $y = \exp(0.0123x + 2.83)$, $R^2 = 0.719$; $p < 0.005$ for all slopes. **(C)** *Ecklonia*: NSW, $y = 0.000156x + 0.907$, $R^2 = 0.00481$; TAS, $y = 0.000136x + 0.358$, $R^2 = 0.00112$; **(D)** *Phyllospora*: NSW, $y = -0.00204x + 0.828$, $R^2 = 0.0171$; TAS, $y = -0.00288x + 1.62$, $R^2 = 0.0132$; $p > 0.4$ for all slopes. Dashed line in **(C, D)** indicates the point of zero net growth.

3.4.2 Photosynthetic characteristics

RLCs measured *in situ* revealed differences in the photophysiology of *Phyllospora* and *Ecklonia* at Nelson Bay, NSW and Fortescue Bay, Tasmania. Seaweeds in NSW were characterised by higher photosynthetic capacity ($rETR_{max}$) than their Tasmanian conspecifics (although not significantly greater for *Phyllospora*), while NSW *Ecklonia* also had a greater maximum quantum yield (F_v/F_m) than Tasmanian *Ecklonia*. Strong evidence of transplant stress was evident at t_0 (likely light stress induced by transport and surface handling of thalli), indicated by increased saturating light intensity (E_k) and reduced maximum quantum yield and photosynthetic capacity (Fig. 3.2). Initial differences in photosynthetic characteristics of NSW and Tasmanian seaweeds (both species) rapidly converged following transplant, and were indistinguishable 23 days after transplant occurred (Table 3.2; Fig.3. 2). Over the remaining 100 days, all seaweeds responded in a similar manner to seasonal environmental changes, with F_v/F_m increasing, and $rETR_{max}$ and E_k declining from March through June (Fig. 3.2; Fig. 3.3).

3.4.3 Pigment content

Tasmanian seaweeds initially contained a greater total pigment pool than NSW seaweeds, although concentrations of individual pigments were similar between thalli at each location (except fucoxanthin, which was present in greater concentrations in Tasmanian *Ecklonia;* Fig. 3.4). Chlorophyll *a* and fucoxanthin increased in all seaweeds over the duration of the experiment, while chlorophyll *c* increased in Tasmanian *Phyllospora* and declined in NSW thalli (significant Time × Origin interaction; Table 3.2; Fig. 3.4). While NSW and Tasmanian seaweeds generally responded in a similar manner over time by increasing overall pigment content (except for chlorophyll *c* in NSW *Phyllospora*), Tasmanian thalli retained a greater total pigment pool (chl *a* + chl *c* + fucoxanthin) at the end of the experiment (~15% and 25% greater in Tasmanian *Phyllospora* and *Ecklonia*, respectively; Fig. 3.4).

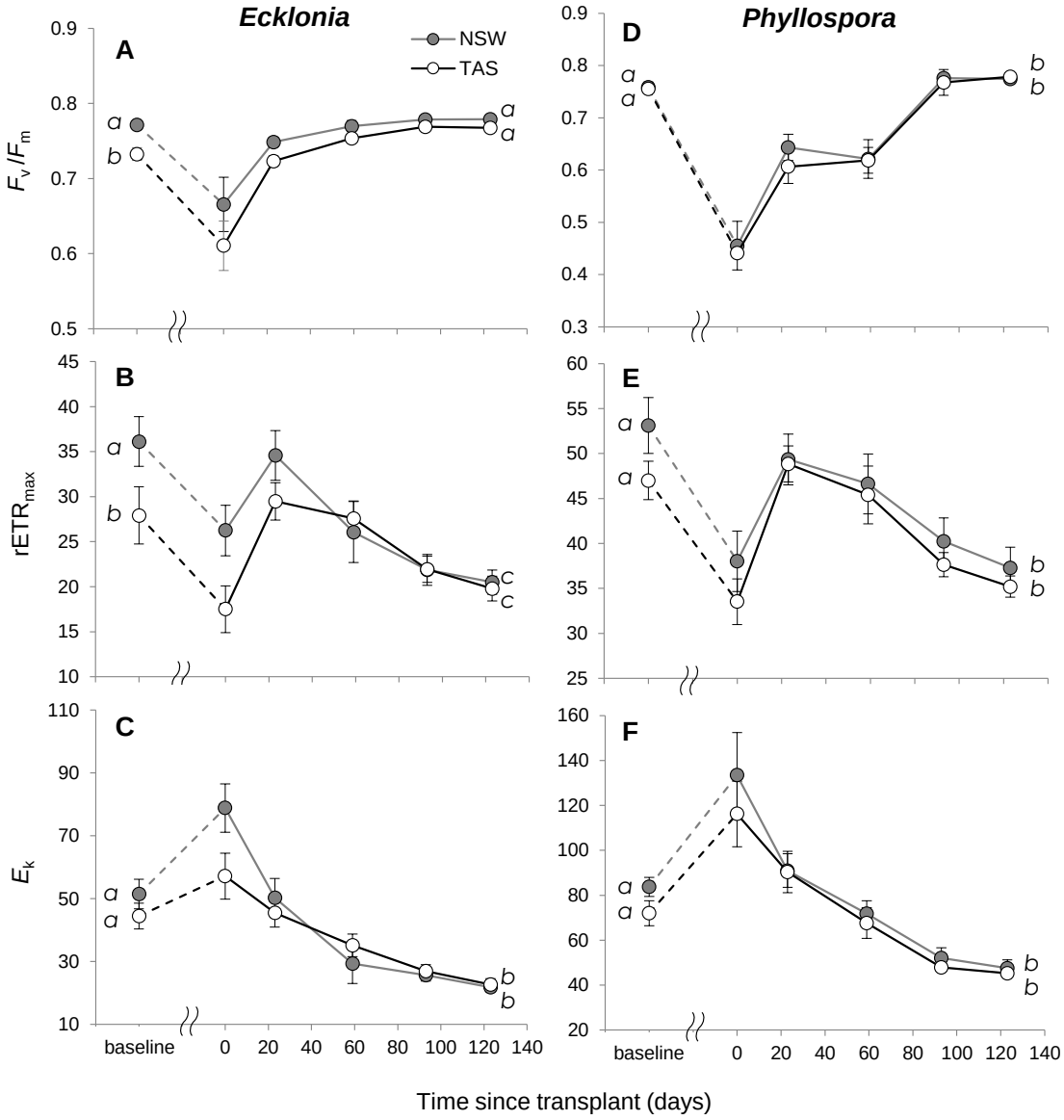


Figure 3.2 Photosynthetic characteristics of juvenile seaweeds originating from NSW (solid circles, grey line) and Tasmania (open circles, black line) in the 123 days following transplantation to Tasmania, as derived from Rapid Light Curves measured by PAM fluorometry of dark-acclimated tissue: **(A-C),** *Ecklonia radiata;* **(D-F)**, *Phyllospora comosa.* 'Baseline' values (to the left of the axis break) represent samples taken at the time of seaweed collection from unmanipulated plants growing *in situ* in NSW and Tasmania. Values plotted are means of parameters (n = 10) ± standard error bars. Letters beside start and end values indicate significance of differences between parameters determined by Tukey's HSD test.

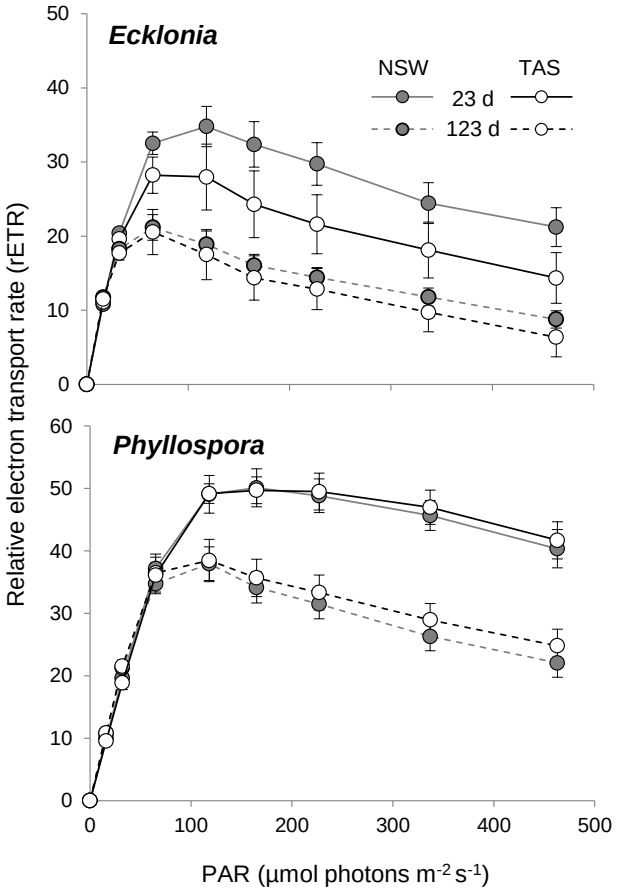


Figure 3.3 Rapid Light Curves (RLCs) of juvenile *Ecklonia radiata* and *Phyllospora comosa* originating from NSW (closed circles) and Tasmania (open circles) measured by PAM fluorometry on dark-acclimated tissue. Curves show thalli at the "beginning" and "end" (123 days following transplant; dashed lines) of transplantation to Tasmania. Note that RLCs are shown from the first sampling occasion following transplantation (t = 23 d) as thalli sampled at t_0 were subject to handling stress (Fig. 3.2). Values plotted are means (n = 10) ± standard error bars.

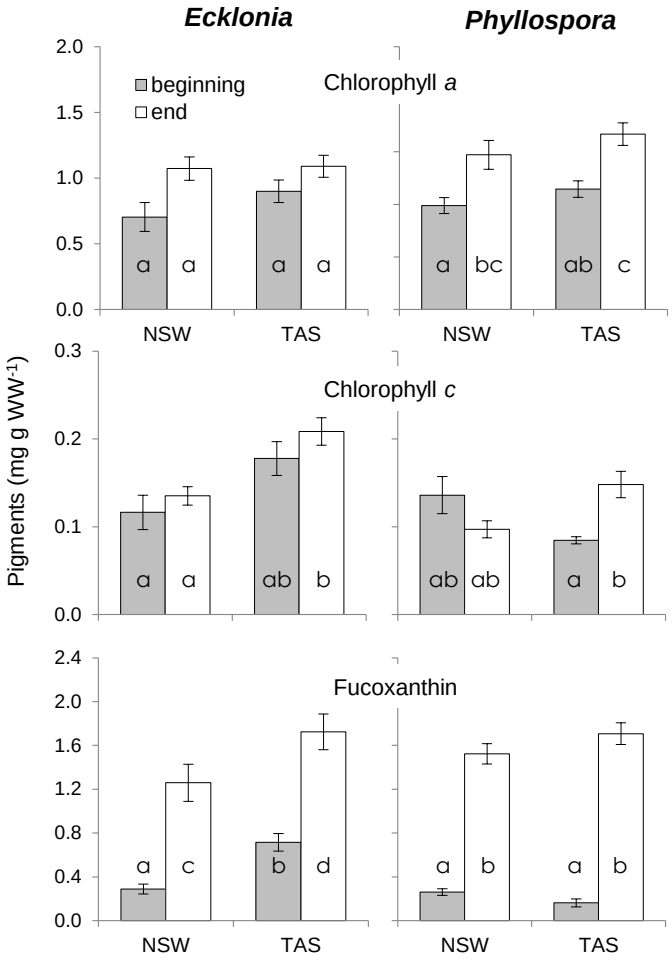


Figure 3.4 Pigment composition of juvenile *Ecklonia radiata* and *Phyllospora comosa* derived from NSW and Tasmania before (*in situ*) and 123 days following transplantation NSW-TAS and TAS-TAS. Values plotted are mean pigment concentrations (n = 10) ± standard error bars. Letters on bars indicate significance of differences between parameters determined by Tukey's HSD test.

3.4.4 Tissue chemistry, isotopic signatures, and nucleic acids

At the time of initial field collection, NSW seaweeds had higher C:N ratios (driven predominantly by a lower % N) and a greater proportion of the heavy carbon isotope $\delta^{13}C$ (although only significantly so in *Phyllospora*) relative to their Tasmanian conspecifics. The fraction of $\delta^{15}N$ was consistent across both species and origins (Table 3.2; Fig. 3.5). In general, *Phyllospora* had lower N content (and thus greater C:N ratios) and $\delta^{13}C$ values than *Ecklonia* (Fig. 3.5). Seaweeds from both collection sites demonstrated similar increases in % C and $\delta^{15}N$ over the duration of transplant, whereas changes in % N showed no consistent pattern across species or origins. C:N ratios inversely followed patterns of % N (i.e. C:N ratios were driven predominantly by % N, not % C). *Ecklonia* maintained a consistent fraction of $\delta^{13}C$ throughout the transplant, whereas *Phyllospora* demonstrated both increased (less negative; Tasmanian thalli) and decreased (more negative; NSW thalli) $\delta^{13}C$ values (Table 3.2; Fig. 3.5).

Seaweeds *in situ* in NSW and Tasmania had a similar content of both RNA and DNA (and thus RNA:DNA ratios; Table 3.2; Fig. 3.6). Four months following transplant, all thalli showed a common trend of decreased DNA concentrations relative to *in situ* values, while *Phyllospora* from both origins exhibited slight (although not significant) increases in RNA content. This resulted in increased RNA:DNA ratios across both species irrespective of origin, but this increase was only significant for *Phyllospora* (Table 3.2; Fig. 3.6).

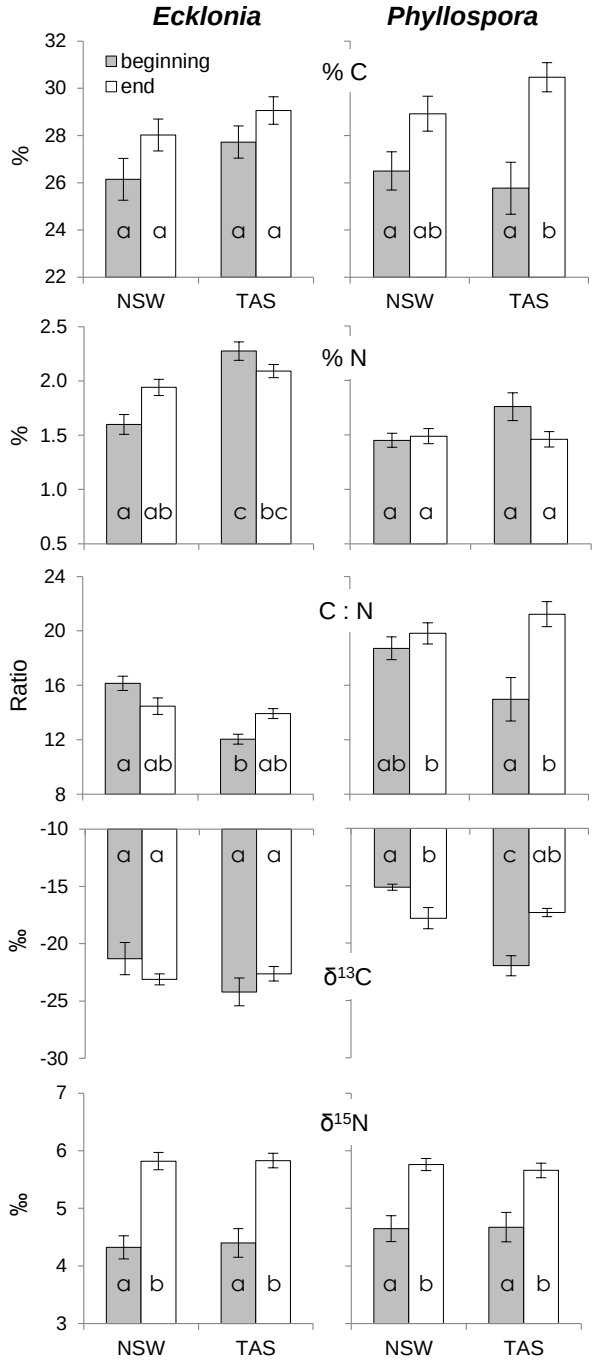


Figure 3.5 Carbon (C) and nitrogen (N) characteristics of juvenile *Ecklonia radiata* and *Phyllospora comosa* derived from NSW and Tasmania before (*in situ*) and 123 days following transplantation NSW-TAS and TAS-TAS. Plots show % C, % N, C:N, $\delta^{13}C$, and $\delta^{15}N$. Values plotted are means of measurements (n = 10 minimum) ± standard error bars. Letters on bars indicate significance of differences between parameters determined by Tukey's HSD test.

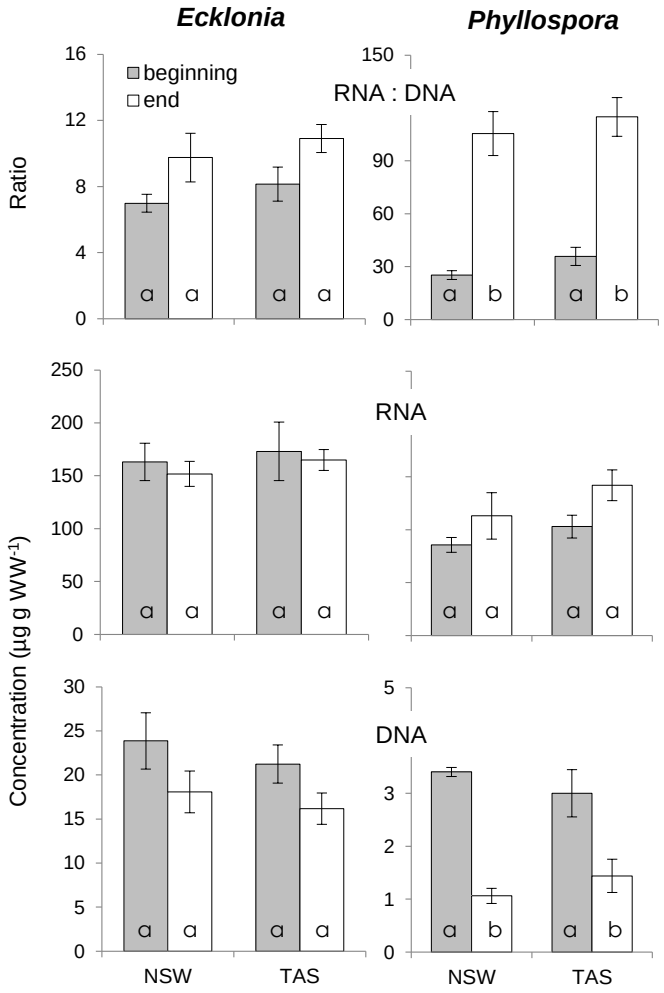


Figure 3.6 Nucleic acid content of juvenile *Ecklonia radiata* and *Phyllospora comosa* derived from NSW and Tasmania before (*in situ*) and 123 days following transplantation NSW-TAS and TAS-TAS. Plots show absolute concentrations of RNA and DNA, and RNA:DNA ratios. Values plotted are means of measurements (n = 10 minimum) ± standard error bars. Letters on bars indicate significance of differences between parameters determined by Tukey's HSD test.

3.4.5 Physical environment

Water temperature and irradiance at Fortescue Bay showed strong seasonal patterns at the transplant site in Fortescue Bay, with temperature decreasing from ~18.7 °C to 12.8 °C, and light from ~7.0 to 0.5 mol photons $m^{-2}day^{-1}$ between 29th February and 30th June 2012 (duration of transplant; Fig. 3.7). Water temperature decreased relatively uniformly over this period, whereas irradiance plateaued at a low level (~0.5 mol photons m^{-2} day^{-1}) in late April and remained relatively consistent for the remaining two months of experimental monitoring (Fig. 3.7).

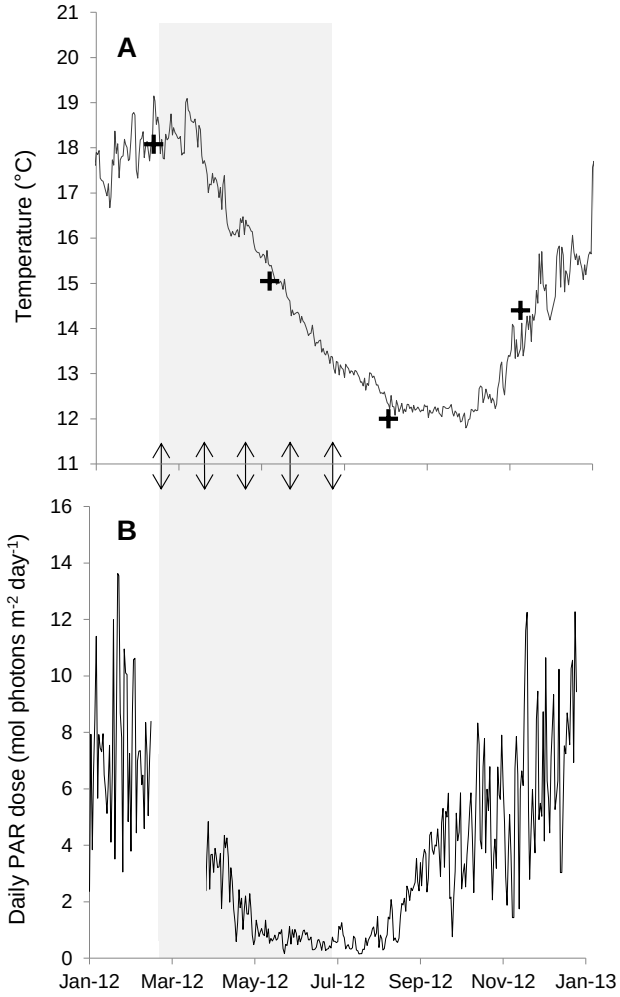


Figure 3.7 Environmental characteristics measured at the site of seaweed transplant in Fortescue Bay, Tasmania. The period of the transplant experiment is indicated in grey. **(A)** water temperature, measured by an IMOS monitoring buoy at Maria Island, Tasmania (line) and *in situ* SONDE profiling (+); **(B)** total daily PAR (plotted as a 2-day average) measured by a subsurface light meter deployed at depth of 10 m. Arrows (↕) show sampling occasions when growth and PAM fluorometric measurements were made (including the initial transplantation of thalli and final measurements).

3.5 Discussion

We found significant changes in a number of ecophysiological characteristics of *Ecklonia* and *Phyllospora* transplanted from Nelson Bay, NSW to Fortescue Bay, Tasmania. However, 'local' transplant controls (removed from Fortescue Bay and re-planted to the same site) demonstrated a similar suite of changes over the four months following transplantation, indicating that (1) physiological characteristics of NSW and Tasmania seaweeds are highly plastic and predominantly under environmental control; and (2) the physiology of *Ecklonia* and *Phyllospora* is strongly seasonally influenced. We did not monitor unmanipulated seaweeds *in situ* over this same period, as we assumed that changes induced in 'local' transplant controls reflected fluctuations in response to environmental conditions. Therefore, any divergence in the physiological characteristics of NSW and Tasmanian seaweeds is interpreted to represent inherent ecophysiological differences between high- and low-latitude seaweeds.

3.5.1 Physiological convergence of translocated and 'home' thalli

The majority of ecophysiological characteristics of NSW and Tasmanian seaweeds responded in a similar manner to environmental change. Values of $rETR_{max}$, fucoxanthin concentrations (for *Ecklonia*), % N (and thus C:N) and $\delta^{13}C$ varied initially in both species depending on the seaweed's origin, as did F_v/F_m in *Ecklonia*. However (within a species), these characteristics converged over the duration of the experiment (as well as changing seasonally) such that low- and high-latitude seaweeds ultimately displayed a very similar suite of physiological characteristics. In general (with the notable exception of *Macrocystis pyrifera*; Wheeler & Srivastava 1984, Shivji 1985, Tussenbroek 1989), macroalgae are capable of highly efficient uptake and storage of N and other nutrients during periods of high environmental availability (Fujita & Edwards 1989, Pedersen & Borum 1996). While NSW seaweeds maintained a consistent % N or a slight accumulation of N following transplantation, Tasmanian thalli became slightly depleted in N despite increased environmental availability of dissolved inorganic nitrogen (DIN; see Chapter 4 of this thesis). Similarly for $\delta^{13}C$, NSW and Tasmanian *Phyllospora* demonstrated variation in $\delta^{13}C$ values which indicates different sources of inorganic carbon used for photosynthesis (macroalgae restricted to CO_2 use typically have $\delta^{13}C$ values below -29‰, while those that also utilise HCO_3^- via active carbon concentrating mechanisms [CCMs] typically have values > -25‰; Raven et al. 2000). A

typical seasonal change in patterns of C utilisation is increased diffusive uptake of CO_2 through autumn-winter in response to lowered temperature and irradiance when elimination of competition between O_2 and CO_2 at the RuBPCase active site becomes less critical (Bidwell & McLachlan 1985). Thus, NSW *Phyllospora* behaved as expected under conditions of decreased carbon demand by becoming more depleted in $\delta^{13}C$ (i.e. decreased their use of CCMs), while Tasmanian *Phyllospora* became more enriched in $\delta^{13}C$, indicating a greater utilisation of energetically-demanding CCMs (Raven & Beardall 2014) and resulting in an associated increase in % C (and hence C:N ratios). The discrepancies between the C and N dynamics of juvenile *Ecklonia* and *Phyllospora* from different origins may be due to NSW thalli being larger (and possibly in different settlement stages) than Tasmanian individuals and thus having more effective mechanisms of N uptake and/or storage (Henley & Dunton 1995) and CCM efficiency. The ultimate convergence in % N, C:N and $\delta^{13}C$ between individuals of both species indicates a similar inherent N storage capacity of high- and low-latitude individuals, and the final $\delta^{13}C$ values of *Ecklonia* and *Phyllospora* were similar to values measured in adult thalli (-19‰ and -16‰ respectively; Chapter 4)

Photosynthetic characteristics that initially differed between *in situ* populations ($rETR_{max}$, and F_v/F_m for *Ecklonia*) also converged over the duration of transplant, indicating a strong environmental influence on photophysiology. The rate of electron transport through PSII (rETR) typically varies with temperature as it is regulated by the activity of Calvin cycle enzymes (Raven & Geider 2003, Hurd et al. 2014). Thus, under the same temperature regime, NSW and Tasmanian seaweeds showed similar rates of enzymatic processes. Similarly, photosynthetic efficiency (F_v/F_m) – which often declines under high irradiance (Ralph & Burchett 1995, Cordi et al. 1997, Gévaert et al. 2002) – was initially lower in Tasmanian *Ecklonia* (likely due to photoinhibition in February when irradiance was high) but converged over the experimental duration when seaweeds were exposed to the same light regime. Of all measured ecophysiological characteristics, the concentration of fucoxanthin (in *Ecklonia*) was the only trait to not totally converge between NSW and Tasmanian seaweeds over the four months following transplantation. However, given that Tasmanian *Ecklonia* initially contained approximately twice the concentration of fucoxanthin as NSW thalli, the experimental duration may have been insufficient time for convergence to occur.

3.5.2 Seasonal changes in ecophysiology

While the primary objective of this study was to determine the extent of environmentally-driven plasticity in the ecophysiology of NSW and Tasmania seaweeds, the four months following transplantation (from 28th February to 30th June 2012) also represented a marked gradient of environmental change. Total daily irradiance over this period decreased by more than an order of magnitude, and water temperature dropped by almost 5 °C. Nutrient levels also varied seasonally, with nitrate concentrations increasing from undetectably low levels (< 0.5 µM) at the time of transplant to > 3 µM (Chapter 4). Simultaneous changes in multiple abiotic factors made it difficult to isolate the effects of individual factors on seaweed physiology. Nonetheless, over the duration of the experiment we observed several general patterns: an increased concentration of photosynthetic pigments and associated increase in photosynthetic efficiency (F_v/F_m), a reduction in maximum photosynthetic capacity ($rETR_{max}$) and sub-saturating irradiance (E_k), and an increase in the tissue fraction of the heavy N isotope $\delta^{15}N$.

An increased concentration of chlorophyll *a*, demonstrated by *Ecklonia* and *Phyllospora* from both origins, typically indicates an increased number of reaction centres per thallus area (Ramus 1981, Fairhead & Cheshire 2004b), while increases in fucoxanthin were likely associated with increased allocation of antennae pigments to individual reaction centres and enhanced photon absorption of light harvesting complexes (Ramus et al. 1976). Although Fairhead & Cheshire (2004) found no direct relationship between irradiance and fucoxanthin concentration in adult *E. radiata,* we suggest that fucoxanthin may play a light harvesting (Gévaert et al. 2002, Fairhead & Cheshire 2004b) rather than photoprotective (Ramus et al. 1977, Stengel & Dring 1998) role in juvenile *Ecklonia* and *Phyllospora*, as decreasing irradiance during winter was associated with large increases (2 to 10-fold) in fucoxanthin concentration. Although changes in chlorophyll *c* were inconsistent across species and seaweed origins, this pigment comprised <5% of the total pigment pool and likely plays a relatively minor role in light harvesting.

Some fucoids (Niemeck & Mathieson 1978) and laminarians (Davison & Davidson 1987, Sakanishi et al. 1989, Fairhead & Cheshire 2004b) are capable of adjusting their light harvesting efficiency and specific activity of Calvin cycle enzymes to compensate for reduced winter temperature and irradiance, resulting in an increased efficiency of PSII

(F_v/F_m), and a reduction in sub-saturating photon irradiance (E_k; Cheshire et al. 1996, Fairhead & Cheshire 2004). The increase in photosynthetic efficiency of *Ecklonia* and *Phyllospora* we observed in response to decreasing temperature and irradiance over winter indicates that these species are (at least partially) capable of compensating for seasonal fluctuations in environmental conditions, although not to the same extent as has been observed in adult *Saccharina latissima* (Davison & Davidson 1987), *E. cava* (Sakanishi et al. 1989) and *E. radiata* (Fairhead & Cheshire 2004b). Juvenile *Ecklonia* and *Phyllospora* may lack sufficient physiological plasticity to fully respond to seasonal environmental changes of this magnitude, or the extent of photosynthetic adjustment (via alteration of the number of reaction centres and/or absorptional cross-section of PSII; Falowski & Raven 1997) required to maximise photosynthetic rates under fluctuating conditions may be energetically unfeasible. Photosynthetic compensation in juvenile *Ecklonia* and *Phyllospora* may therefore represent a trade-off between diverting energy resources to optimise photosynthetic efficiency, while maintaining sufficient growth rates to allow young thalli to remain competitive beneath canopies of adult seaweed.

The isotopic composition of macroalgal tissues typically reflects the composition of seawater, as N, P and other nutrients are absorbed directly from the water column (Lehvo et al. 2005). We observed similar values of the heavy nitrogen isotope $\delta^{15}N$ in both *Ecklonia* and *Phyllospora* measured *in situ* in February (mean ± SE ~4.4 ± 0.2 and ~4.7 ± 0.3 for *Ecklonia* and *Phyllospora* respectively at both sites), indicating similar DIN characteristics of summer coastal water masses near Nelson Bay, NSW and in Fortescue Bay, Tasmania. Sources of DIN can include remineralized NH_3, upwelled NO_3^-, and additional DIN from terrestrial runoff (Sigman et al. 2000). The increase in seaweed tissue $\delta^{15}N$ values from summer through to winter reflect the reduced influence of the warm NO_3^- and NH_3 depleted East Australian Current, and flushing of Fortescue Bay with Southern Ocean water which is typically more isotopically enriched in ^{15}N (Sigman et al. 1999). Importantly, the consistent changes in N-isotope composition of *Ecklonia* and *Phyllospora* from both latitudes, while clearly reflecting shared water column geochemistry (Fourqurean et al. 1997), indicates consistency in the species of N absorbed.

3.5.3 Growth patterns, and decoupling of growth from RNA:DNA ratios

Growth is frequently used as an indicator of physiological success as it is the end product of multiple simultaneously operating biochemical processes (Hasanuzzaman et al. 2013). Given the vastly different environments from which low- and high-latitude seaweeds originated, it could reasonably be expected that Tasmanian thalli in their 'home' environment would outperform NSW individuals due to local adaptation (e.g. Waser & Price 1985, Joshi et al. 2001, Santamaría et al. 2003). Surprisingly however, NSW and Tasmanian *Phyllospora* exhibited remarkably similar growth patterns, while transplanted NSW *Ecklonia* slightly outperformed Tasmanian individuals (although not when corrected for initial size). Moreover, growth patterns were unrelated to other measured physiological parameters. This suggests either a lag between changes in photophysiology or nutrient acquisition and expression of growth, and/or that growth is the product of a complex interaction between multiple (potentially unmeasured) processes. Alternatively, growth may have occurred through increases in tissue thickness (i.e. change in biomass; Fowler-Walker et al. 2006, Enríquez et al. 2009) which would not have been detected by our non-destructive measurements.

The relative growth estimates of 0.5-1.5% day^{-1} observed here are modest compared to measurements of laminarian and fucoid growth reported in other field studies (Sidman 1983, Dean & Jacobsen 1984) but are likely to be relatively conservative as net growth incorporates losses to necrosis, grazing and erosion. A potential alternative to direct measurements of growth is the use of RNA:DNA ratios as an index of an organism's 'growth potential' (Holm-Hansen 1969, Dortch et al. 1983). RNA:DNA ratios have been successfully used as a proxy for growth in larval fish (e.g. Buckley 1984, Buckley et al. 1999), zooplankton (e.g. Sutcliffe 1965, Sutcliffe 1969, Dortch et al. 1983, Acharya et al. 2004) and higher plants (e.g. Matzek & Vitousek 2009, Reef et al. 2010b). However, we found no link between growth rate and RNA:DNA ratios in either *Ecklonia* or *Phyllospora*. This decoupling of growth from RNA:DNA ratios supports the observations of Reef et al. (2012) with tropical seaweeds (*Caulerpa*, *Laurencia* and *Sargassum* spp.) and a recent laboratory study of *P. comosa* (Chapter 2) which currently provide the only studies (to our knowledge) to investigate the link between RNA:DNA ratios and growth of macroalgae. We could not test for the correlations between RNA:DNA ratios and growth of individuals as thalli were not uniquely identified when they were destructively sampled. However, mean RNA concentrations in *Ecklonia* and *Phyllospora* four months following transplantation were largely unchanged

from *in situ* values, and we found no evidence for increased rates of RNA synthesis associated with rapid growth. The high RNA:DNA ratios (described for *Phyllospora* from both origins) were driven by a decrease in the absolute concentrations of DNA rather than increased RNA. DNA concentration is predominantly regulated by cell packing density (Dortch et al. 1983) hence the decreased DNA concentration we observed in *Phyllospora* may be the result of increased cell size and/or thickening of cell walls (Kraemer & Chapman 1991) not detectable by our growth measurements. Importantly, the similar nature of patterns in RNA:DNA ratios across all groups indicate that (1) RNA:DNA ratios are a poor indicator of growth in these seaweed species (as RNA:DNA ratios increased significantly in *Phyllospora* while growth rates remained constant); and (2) RNA synthesis is likely associated with non-growth related cellular activity such as repair (Kirst & Wiencke 1995), protective metabolic processes (e.g. Britto et al. 2001), or synthesis of antioxidant defences (Davison & Davidson 1987, Sweetlove et al. 2002, Hasanuzzaman et al. 2013).

3.5.4 Ecological implications of physiological plasticity in *Ecklonia* and *Phyllospora*

Most ecophysiological characteristics of *Ecklonia* and *Phyllospora* convergenced following four months of growth in Tasmania, indicating that the physiology of these species is predominantly under environmental control. This suggests that differences in the photophysiology and tissue chemistry of seaweeds *in situ* in Nelson Bay and Fortescue Bay is due to simple temperature- or light-driven regulation of enzymatic processes, or represents dynamic acclimation to environmental conditions by net molecular synthesis or breakdown (Raven & Geider 2003). Given the tendency for localised recruitment in *Ecklonia* and *Phyllospora* and the inherently restricted nature of gene flow across their respective distributions (Coleman et al. 2009, Coleman & Kelaher 2009), genetic divergence across the range of these species is likely relatively high. Therefore, while warm-adapted (NSW) *Ecklonia* and *Phyllospora* clearly have the inherent physiological capacity to acclimatise within their genetic constraints to environmental conditions characteristic of higher latitudes (Tasmania), this does not necessarily imply the converse is true. That is, under predicted levels of climate change where current environmental conditions at low latitudes are spatially extrapolated to higher latitudes (the 'climate envelope' concept; Pearson et al. 2004), physiological plasticity will not necessarily ensure survival of Tasmanian seaweeds under warmer oligotrophic conditions. As this study design did not incorporate site replication,

unmanipulated controls, or a full reciprocal transplant, caution must be exercised in interpreting these results across a broad spatial context as local factors (such as wave exposure, grazer pressure, interspecific competition) may influence physiological success. Therefore, while we do not attempt to predict the performance of *Ecklonia* and *Phyllospora* under future climate change scenarios, this study provides an important first step in exploring the extent of environmental influences on phenotypic trait expression.

3.5.5 Conclusions

The results of this experiment indicate that juvenile *Ecklonia* and *Phyllospora* have highly plastic photophysiology, growth, nutrient acquisition and biochemical processes, demonstrating that environmental (non-genetic) regulation largely determines trait expression as has been reported for macroalgae elsewhere (e.g. Santamaría et al. 2003, Fairhead & Cheshire 2004b, Jormalainen & Honkanen 2004, Wright et al. 2004). While northern and southern populations of both species display different photophysiological characteristics (including pigment composition), tissue chemistry, and morphology (not directly quantified here, but see Mabin et al. [2013] for *Ecklonia*) when measured *in situ*, we observed convergence in most measured physiological characteristics over the four months following transplantation. Seasonal responses of *Phyllospora* and *Ecklonia* matched those typically reported in temperate macroalgae elsewhere, providing evidence for acclimation via adjustment of the photosynthetic apparatus which presumably permits optimisation of growth and photosynthetic performance across a wide range of temperature and irradiance. We found no evidence for reduced performance of seaweeds originating from 'warm' conditions (low latitude) when transplanted to 'cool' conditions (high latitude), with NSW *Ecklonia* growing more rapidly than Tasmanian *Ecklonia* despite temperatures 3-4 °C below the normal winter minima experienced by these individuals. The capacity of *Phyllospora* and *Ecklonia* to acclimate to novel environmental conditions may indicate a potential tolerance to future climatic change (which is particularly important if gene flow in these species is as restricted as has been suggested; Coleman et al. 2009, Coleman & Kelaher 2009). However, determination of phenotypic plasticity in an ecological context requires a full reciprocal transplantation of seaweeds from cool to warm coasts and vice versa. Further work should attempt to determine the upper thermal tolerance of *Ecklonia* and *Phyllospora* across their respective latitudinal distributions to facilitate predictions for the future of these species under a changing climate.

Chapter 4 Latitudinal and depth variation in the ecophysiology of three habitat-forming seaweeds: the importance of ocean temperature and summer irradiance

4.1 Abstract

Seasonal patterns in the *in situ* ecophysiology of the common habitat-forming seaweeds *Ecklonia radiata*, *Phyllospora comosa*, and *Macrocystis pyrifera* were investigated at different latitudes and depths in southeastern Australia. This region is warming at four times the global average rate in combination with climate change-driven oligotrophy. The Growth Rate Hypothesis (GRH) predicts that low nutrient stress will impact more severely on seaweeds with increasing latitude and depth as reduced temperatures and light availability compresses growth seasons. We used multiple performance indicators (photosynthetic characteristics, pigment content, chemical composition, stable isotopes, nucleic acids) to assess the ecophysiology of seaweeds near the northern and southern margins of their range, along a depth gradient (*E. radiata* only), over a two year period (September 2010 – August 2012). All three species were strongly influenced by seasonal fluctuations in the physical environment (predominantly temperature and irradiance), but the nature of these patterns varied with both depth and latitude. Nitrogen limitation occurred in all species over summer when ambient nitrate levels were low, but decreased in severity with increasing depth. No link was detected between RNA synthesis (or RNA:DNA ratios) and ambient nutrient availability or any other physiological indicator, suggesting the GRH is not supported in these seaweeds. *Ecklonia radiata* and *P. comosa* at high latitude indicated significant stress over summer due to high irradiance despite demonstrating photophysiological compensation, but lower latitude and deeper thalli showed no signs of light stress. In the context of climate change for this region, we predict that *Ecklonia* and *Phyllospora* will be affected similarly across their latitudinal range by increased temperature and reduced nutrients, but these stressors may act synergistically with high summer irradiance so that seaweeds at high latitudes will be more severely impacted than is typically predicted. For seaweeds spanning a large depth distribution, deep water populations are likely to provide a refugia from increasingly stressful environmental conditions driven by changing climate.

4.2 Introduction

Species with broad geographical distributions commonly span large environmental gradients and typically exhibit a high degree of variability in phenology, physiology, and life history traits (e.g. Zhang & M.J. 1994, Li et al. 1998, Jensen et al. 2000, Bertness & Ewanchuk 2002, Santamaría et al. 2003). The extent of this variation can often be related to the distance of an individual from the edge of its physiological tolerance (Lawton 1993, Vucetich & Waite 2003), how recently the species has established in a particular region (i.e. timescale over which adaptive specialisation may occur; reviewed by Crooks et al. 1999), or the degree of environmental variation across the species' distribution. While individuals in rear edge (low latitude) populations are often highly specialised to perform in environments unsuitable for populations from other parts of a species' range (Nilsson-Örtman et al. 2012), individuals in lower range edge (high latitude) populations are typically generalists and exhibit considerable plasticity (Santamaría et al. 2003). Thus, while a species may be successful over a wide range of environmental conditions (Santamaría et al. 2003), the capacity of individual genotypes to perform well across this range is usually considerably more limited (DeWit et al. 1998). Examining relationships between the performance of a species across its geographic distribution and latitudinal clines in environmental factors can provide insight into how changes in climate are likely to affect ecological processes and patterns (Bertness & Ewanchuk 2002).

Latitudinal variability in morphology, ecology, or physiological performance is usually particularly pronounced in sessile species where individuals are unable to move along environmental gradients. Indeed, worldwide patterns in vegetation zones and the distribution of photosynthetic organisms in terrestrial (e.g. Walter 1973, Woodward 1990) and aquatic (Santelices & Marquet 1998, Wernberg et al. 2010) systems are driven largely by clines in abiotic factors. An important ecological theory in the context of physiological performance along latitudinal (and environmental) gradients is the Growth Rate Hypothesis (GRH), developed by Elser et al. (2003), which predicts that autotrophs with higher growth rates will be selected for at higher latitudes to compensate for a growth season shortened by low temperature and reduced light availability. Under ecological stoichiometry, an autotroph's performance depends on its capacity to maintain tissue C:N:P in stoichiometrically balanced ratios (Sterner & Elser 2002). As growth depends on the allocation of resources (primarily P and N) to ribosomal RNA, rapidly growing autotrophs (at higher latitudes) will have higher

nutrient requirements (particularly P) associated with an increased demand for protein synthesis. A direct consequence of higher nutrient demands and compressed growth seasons is an increased susceptibility to nutrient limitation at high latitudes due to the greater demand placed on resources by rapid growth (Kerkhoff et al. 2005, Lovelock et al. 2007).

In the marine environment, seaweeds (like terrestrial plants) show a high degree of variation in morphology (Wernberg et al. 2003, Wernberg & Thomsen 2005, Fowler-Walker et al. 2006, Stewart 2006) and physiology (Steneck et al. 2002, Wernberg et al. 2010) along environmental gradients. Unlike terrestrial plants, however, seaweeds are thought to have relatively low genetic variance for key life-history traits (e.g. Jormalainen & Honkanen 2004, Wright et al. 2004) which has led to the development of 'general-purpose' genotypes capable of maintaining high fitness over a broad range of environmental conditions (e.g. Steneck et al. 2002, Fowler-Walker et al. 2006, Stewart 2006, Wernberg et al. 2010), but often at a cost of reduced fitness to individuals (Wernberg et al. 2010). This is particularly important in the context of future climate- and anthropogenic-driven changes, where predicted ocean warming (e.g. Kirkman 1984, Wernberg et al. 2010, Wernberg et al. 2011a) may act synergistically with other stressors such as nutrient depletion (Hurd et al. 2014), eutrophication (Russell & Connell 2007), ocean acidification (Connell & Russell 2010, Harley et al. 2012), increased frequency of storm events (Harley et al. 2006) and shifts in herbivore abundance (Ling 2008) to erode the resilience of seaweed beds via direct physiological effects (Wernberg et al. 2011a) and reduced recruitment (Breeman 1990, Mabin et al. 2013, Mohring et al. 2013a).

The shallow rocky reefs of southeastern Australia are home to some of the most productive and diverse seaweed beds in the world, and are dominated by the canopy-forming phaeophytes *Ecklonia radiata, Macrocystis pyrifera, Durvillaea potatorum* and *Phyllospora comosa* (Millar 2007). This coastline is also warming at a rate nearly four times the global average (Ridgway 2007) as a result of larger and more frequent incursions of warm East Australian Current (EAC) water off eastern Tasmania (Cai 2006, Ridgway 2007). The EAC water is nutrient-poor relative to the sub-Antarctic water masses that have, until recently (last 2-3 decades), dominated waters off the east coast of Tasmania (Harris et al. 1987, Ridgway 2007). Climate change-driven range contractions have already been documented in *E. radiata* (Wernberg et al. 2011b) and the coverage of dense surface canopies of *M. pyrifera* in Tasmania have declined by ~95% over the last 3-4 decades (Johnson et al. 2011), while

discontinuities in *P. comosa* distribution have formed along large stretches of urbanized New South Wales coastline (Coleman et al. 2008, Coleman & Kelaher 2009),

Given the capacity of seaweed to respond to annual cycles of temperature that are greater in range than the changes predicted by global warming scenarios (IPCC 2013), climate change in southeastern Australia is unlikely to lead to the complete removal of seaweeds from this region. However, reduced resilience may exacerbate other anthropogenic perturbations and stressors, and be particularly critical at high latitudes where the GRH predicts disproportionately severe effects of climate-driven stressors (particularly oligotrophy) on seaweeds due to the faster instantaneous growth rates necessitated by compressed growth seasons. The GRH also predicts that nutrient depletion will have increasingly severe negative impacts with increasing depth where light is an additional limited resource (Elser et al. 2000, Lovelock et al. 2007), however the applicability of this hypothesis for seaweeds has yet to be rigorously tested (but see Reef et al. 2012, Chapters 2 & 3). While the increase in temperature and decreased nutrients predicted for the waters of southeastern Australia will almost certainly negatively impact the survival, productivity, and physiological performance of seaweeds (Foster & Schiel 1985, Dayton et al. 1999a, Graham et al. 2007b, Mabin et al. 2013), current understanding of how these stressors may differentially affect seaweed along their latitudinal and depth ranges is limited.

Seasonal and/or depth-related phycological studies typically employ *ex situ* measurement techniques (whereby seaweed thalli are collection from a particular location and/or depth and measured under laboratory conditions; e.g. Wheeler et al. 1984, Arnold & Manley 1985, Sakanishi et al. 1989, Flores-Moya et al. 1995, Serisawa et al. 2001, Andersen et al. 2013). These studies can be difficult to interpret due to the artificial nature of laboratory conditions, including reduced variability in irradiance and/or altered spectral quality (Kirk 1994, Kübler & Raven 1996), static temperature conditions, reduced flow rates (Cornwall et al. 2014), and "bottle effects" resulting in nutrient depletion (Littler 1979). Recognition of the importance of measuring individuals under natural conditions has resulted in an increasing number of physiological studies based on *in situ* measurements of entire organisms (e.g. Cheshire et al. 1996, Beer et al. 2000, Durako & Kunzelman 2002, Gévaert et al. 2003, Fairhead & Cheshire 2004a, Cornielsen et al. 2007), although these typically utilise single physiological measurements of growth (linear extension, change in tissue weight; e.g. Wheeler & North 1980, Dawes et al. 1994, Altamirano et al. 2000), photosynthesis (PAM fluorometry; Beer &

Bjork 2000, Durako & Kunzelman 2002, Belshe et al. 2008, Ekelund et al. 2008) or productivity (oxygen evolution methods; e.g. Littler 1979, Hanelt et al. 1997, Fairhead & Cheshire 2004a, Fairhead & Cheshire 2004b) to relate seaweed performance to environmental conditions. However, univariate measurements of seaweed can fail to detect organism-scale adaptive responses to changing environmental conditions (Roleda et al. 2004), and cannot provide a comprehensive assessment of an individual's physiological state or facilitate interpretation of the ecological response it underpins. A comprehensive multivariate approach is more likely to provide a robust assessment of physiological change.

This study investigated seasonal patterns over a two year period in the *in situ* physiology of three habitat-forming brown seaweeds (*Ecklonia radiata*, *Phyllospora comosa*, and *Macrocystis pyrifera*, hereafter *Ecklonia*, *Phyllospora*, *Macrocystis*) across latitudinal (*Ecklonia* and *Phyllospora*) and depth (*Ecklonia* only) distributions. A range of performance and ecophysiological indicators (photosynthetic characteristics, pigment concentrations, % C and % N, C:N, absolute RNA and DNA concentrations, RNA:DNA, $\delta^{13}C$ and $\delta^{15}N$) were documented with respect to changes in environmental conditions (temperature, nutrients and irradiance) to assess seasonal patterns in the physiology of these seaweeds across their respective ranges. These patterns were interpreted in the context of potential biogeographic variation in physiological responses to predicted levels of climate change.

4.3 Materials and Methods

4.3.1 Study species and sampling schedule

Ecklonia (Laminariales) is a dominant habitat-forming seaweed with a larger depth (0-60+ m) and latitudinal (27.5-43.5 °S) range than any other canopy-forming species in Australia (Steinberg & Kendrick 1999, Nelson et al. 2014). Adult thalli grow to ~2 m in height, with a single stipe bearing a flattened blade with distinct lateral fronds. In southeastern Australia (from Robe, South Australia to Port Macquarie, NSW; Campbell et al. 2014), *Phyllospora* (Fucales) dominates the shallow high-wave energy zone above the deeper *Ecklonia* band (typically 0-5 m but extending below ~10 m on exposed coastline, with adult thalli growing to a length of 0.5-3 m and comprised of a short stipe bearing multiple primary branches and floats (Campbell et al. 2014). *Macrocystis* (Laminariales) is the world's largest and most rapidly growing seaweed, growing up to 45 m in height with each thalli consisting of a

conical holdfast from which multiple stipes emerge and branch into numerous distal stipes bearing elongated blades and pneumatocysts (Abbott & Hollenberg 1976). *Macrocystis* has a global latitudinal distribution encompassing North America (Alaska to California), South America, South Africa, New Zealand and Southern Australia (Jackson 1982, Santelices & Ojeda 1984, Ladah et al. 1999, Nelson 2013), but recent range contractions of *Macrocystis* in the Australian portion of its distribution have dramatically reduced its cover such that it is now largely restricted to Tasmanian waters.

Ecklonia, *Phyllospora* and *Macrocystis* were sampled approximately quarterly in Tasmania (seasonally) and every ~6 months in NSW (summer and winter; *Ecklonia* and *Phyllospora* only) over a period of two years from September 2010 to August 2012 (Table 4.1). Seaweeds were sampled near Port Stephens in NSW (*Ecklonia*, 32.630594 °S, 152.309621 °E; *Phyllospora*, 32.789370 °S, 152.088040 °E) and Fortescue Bay in Tasmania (43.124710 °S, 147.975580 °E; all species) on open rocky reef subject to flushing by oceanic water. The selected study sites were near the northern (NSW) and southern (Tasmania) limits of the *Ecklonia* and *Phyllospora* range distribution in eastern Australia. *Macrocystis* was only measured in Fortescue Bay, as this species is largely restricted to the East Coast of Tasmania. Both regions were characterised by moderate-relief rocky reef dominated by *Ecklonia*, *Phyllospora* or *Macrocystis* canopy with diverse understorey communities.

Table 4.1 Dates of sampling of *Ecklonia radiata*, *Phyllospora comosa*, and *Macrocystis pyrifera* (Tasmania only). Superscripts show temporally 'matched' sampling occasions used in ANOVA, with * indicating 'summer' sampling, and # indicating 'winter' sampling occasions.

	Sampling dates							
NSW	Sep-10 #	Mar-11 *	Aug-11#	Feb-12 *				
Tasmania	Oct-10 #	Dec-10 *	Jun-11	Sep-11 #	Nov-11	Feb-12 *	May-12	Aug-12

Ecklonia was sampled from two depths (~10 and 30 m) due to its broad depth distribution, while the shallower *Phyllospora* was measured at a single depth (~6 m), and measurements of *Macrocystis* were made in the canopy at a depth of ~1 m below the surface. On each sampling occasion, *in situ* pulse-amplitude-modulated (PAM) fluorometric measurements were taken from a randomly selected set of ten individuals, and tissue collected from each for

analysis of pigment content, tissue chemistry, stable isotopes, and nucleic acids (note that pigment sampling only commenced in June 2011, so pigment data are not available from the first two sampling occasions in either location). See section 4.3.2 *Ecophysiological measurements* for further details on all sampling techniques described above. To minimise within-individual variation in tissue characteristics, the sampled area was standardised as one-third towards the distal end of the primary lamina from the top of the stipe and one-third along the lateral (*Ecklonia*) or branch (*Phyllospora*) at that location, or one-third along a randomly selected blade ~1 m below the surface canopy (for *Macrocystis*). A section (> 60 mm^2) of the thallus was torn from alga using gloved hands and sealed in lightproof containers which were brought to the surface. Tissue was removed from containers at the surface but maintained under low light and rinsed with de-ionized water before being cut into three pieces for separate analyses. All samples were kept on ice in darkness for transport immediately following collection and were refrigerated or frozen (as appropriate) within six hours of collection.

For tissue chemistry and stable isotope analysis (% C, % N, $\delta^{13}C$ and $\delta^{15}N$), 10-20 mm^2 of tissue was rinsed with de-ionized water before freezing at -20 °C for mass spectrometric analysis. A similar sized piece of tissue was removed, rinsed, and stored in 2 ml of RNAlater (Ambion, Inc., Austin, TX), a nontoxic RNA stabilization buffer, for nucleic acid (RNA, DNA) measurements. These samples were refrigerated overnight at 4 °C before being transferred to -80 °C for long-term storage in accordance with the recommendations of Ambion. A third section of tissue 20-40 mm^2 in size was rinsed and stored in darkness at -20 °C for pigment analysis.

4.3.2 Ecophysiological measurements

Elemental composition of tissue

Measurements of tissue chemistry (C and N content) can inform on the dynamics of nutrient uptake and storage by seaweeds, while stable isotopes indicate the environmental source ($\delta^{15}N$) and mechanism ($\delta^{13}C$) of inorganic N and C uptake, respectively. To measure these characteristics, frozen algal samples were thawed, blotted dry and freeze dried for 18 hours before being ground and homogenised. Approximately 5 mg of each dried, powdered sample was weighed into tin cups (Elemental Microanalysis LTD., Okehampton, UK) which were closed prior to analysis. Carbon, nitrogen, and $\delta^{13}C$ and $\delta^{15}N$ content were determined using a

Thermo Gas Chromatograph coupled to a Finnigan Mat Delta S isotope radio mass spectrometer operating in continuous flow mode (analysed by CSIRO, Hobart). Results are presented in standard sigma notation:

$$\delta^{13}\text{C or } \delta^{15}\text{N (‰)} = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \qquad \text{where R} = {}^{13}\text{C}/{}^{12}\text{C or } {}^{15}\text{N}/{}^{14}\text{N}.$$

Externally validated working standards were replaced every 12 samples, with Pee Dee Belemnite (VPDB) used throughout as a standard for carbon and air as a standard for nitrogen. Within-sample variability from a subset of duplicate samples (>20% of total samples extracted) gave the variability for *Ecklonia*, *Phyllospora* and *Macrocystis* (respectively) as follows: carbon: ± 2.2, 1.8 and 3.8%; nitrogen: ± 3.3, 1.9 and 19.2%; C:N: ± 3.5, 2.1 and 18.4%; δ^{13}C: ± 1.6, 2.0 and 2.5%; δ^{15}N: ± 6.3, 3.6 and 9.4% (mean percentage standard error of duplicate samples).

Pulse amplitude modulated (PAM) fluorometry

Electron transport dynamics in photosystem II (PSII) were estimated by variable chlorophyll *a* fluorescence using a blue LED Diving-PAM (Walz, Germany). Prior to fluorescence measurements, epiphyte and bryozoan growth was wiped from the blade surface using gloved fingers. Algal thalli were connected to a "dark leaf clip" (as termed by Walz) to maintain a consistent spacing of the PAM fiber optic from the surface of the tissue and permit fluorescence measurements without ambient light interference. An inbuilt software routine was used to generate rapid light curves (RLCs), where actinic light intensity was increased in eight steps (reaching a maximum of 463 µmol photons m^{-2} s^{-1} at 650 nm) of 10 seconds in duration to measure the effective quantum yield of PSII (ϕ_{PSII}) as a function of irradiance. Relative electron transport rate (rETR) is an approximation of the rate of electrons pumped through the photosynthetic chain (Beer et al. 2001), and can be calculated as ϕ_{PSII} x PAR. Plotting rETR against PAR creates a curve similar to a photosynthesis-irradiance (P-E) curve (although it should not be interpreted as such; see Ralph & Gademann 2005) and provides an assessment of photosynthetic activity.

RLCs were conducted on 15–20 minute dark-acclimated tissue (determined by our pilot studies to be sufficient to allow oxidation of the electron transport chain and relaxation of photoprotective mechanisms; i.e. further dark-acclimation resulted in no further increases in

F_m or F_v/F_m; Appendix A). While ambient light-acclimated RLCs demonstrate the thalli's immediate light history and can be affected by recent canopy shading, water clarity, cloud cover etc., dark-acclimation 'standardises' the pre-manipulation light history and reflects the inherent physiological state of photosystems beyond the immediate light history (Ralph & Gademann 2005). All PAM fluorometry was conducted between 12:00 and 14:00 to minimise potential variability in photosynthetic activity due to diurnal cycles of non-photochemical quenching (e.g. Belshe et al. 2007). Leaf clips were moved 20-30 mm between initiations of each RLC to avoid light stress affecting subsequent fluorometric measurements.

Curves were fitted to raw rETR data to derive the saturating light intensity (E_k) and maximum electron transport rate, or photosynthetic capacity ($rETR_{max}$). The maximum quantum yield, or intrinsic potential efficiency of PSII (F_v/F_m), was also derived from dark-acclimated samples (see Falkowski & Raven 1997). The double exponential decay model of Platt et al (1980) was selected as the model fit because it adequately described both the initial linear response and the region of photoinhibition (Ralph & Gademann 2005):

$$P = P_s\left(1 - exp\left(\frac{-\alpha E_d}{P_s}\right)\right) \times exp\left(\frac{-\beta E_d}{P_s}\right)$$

where P is the photosynthetic rate (here used as rETR), α is the light harvesting efficiency as measured by the initial slope of the RLC before light saturation, E_d is the downwelling actinic irradiance of the PAM's internal halogen light, β is the negative slope of the RLC at high irradiance, and P_s is a scaling factor defined as the maximum potential rETR in the absence of any photoinhibition (if $\beta = 0$, this is equal to $rETR_{max}$). Nonlinear least-squares function using the 'R' software environment (v 3.0.0) was used to fit models, and the parameters $rETR_{max}$ (maximum relative electron transport rate) and E_k were estimated as per the equations given in Ralph and Gademann (2005). The following parameters were used in the curve-fitting routine to ensure convergence: iterations = 100; stepsize = 1/1024; tolerance = 0.00001; initial seed value for P = maximum rETR derived from raw data, α = slope of linear regression fitted to first three points of raw data (typically in the range 0.7 – 1.0).

Pigment extraction

The light absorption capacity of seaweed tissue was estimated by measuring the concentration of photosynthetic pigments. Frozen samples collected for pigment extraction were thawed and blotted dry, then weighed to the nearest 0.01 mg. Approximately 100-150 mg (wet weight) of algal tissue was transferred to a 15 ml vial and extracted into 5 ml of N,N-dimethylformamid (DMF). Processing was conducted rapidly under low light to minimise sample deterioration or change in pigment content resulting from exposure to ambient light. Vials were wrapped in aluminium foil and left in total darkness for 96 hours at -20°C for extraction to take place. Algal tissue was white in colour following DMF extraction, indicating a complete removal of chlorophyll from tissue. Following extraction, 2 ml was removed, centrifuged at 8,000 rpm for 8 minutes and frozen at -80 °C for High Performance Liquid Chromatography (HPLC) analysis.

Centrifuged samples for fucoxanthin determination were measured in 2 µl aliquots using a Waters Acquity H-series UPLC™ (Waters Corporation MA. USA) coupled to a Waters Acquity Photodiode Array Detector (PDA) with an Ethylene Bridged Hybrid (BEH) C-18 column (2.1 x 100 mm x 1.7 micron particles). The mobile phase was a gradient mixture prepared from Merck Chemicals (Merck KGaA, Darmstadt, Germany) with three eluents: 1% acetic acid, acetonitrile, and 80:20 methanol:hexane. Initial conditions were a mixture of 20% acetic acid and 80% acetonitrile for 3.5 minutes, followed by an immediate switch to 80% acetonitrile and 20% methanol:hexane which was then held for a further 5.5 minutes. Samples were left for 3 minutes and allowed to re-equilibrate. Initial calibration of the UV-Vis response at 440 nm for fucoxanthin was carried out on a fresh, accurately prepared standard solution (Sigma Aldrich, Castle Hill, Australia) made up at 1.26 µg mL^{-1} in methanol. The column was held at 35 °C with a flow rate of 0.35 mL $minute^{-1}$ throughout. Chromatograms were extracted at 440 nm from the raw data using Waters TargetLynx V4.1 software. The area beneath the fucoxanthin peak was recorded and converted to mg mL^{-1} fucoxanthin, then to mg g^{-1} wet weight.

Chlorophyll *a* and *c* content was measured from the remaining ~3 mL of DMF extracted sample using a Dynamica HALO RB-10 Spectrophotometer and processed using UV Detective software (v 1.1). Samples were spectrophotometrically measured at the wavelengths 664.5, 631, and 582 nm. Where absorbance was above 1.000, samples were

diluted with DMF until readings dropped below 1.000 in accordance with the manufacturer's recommendations. DMF was used as a blank throughout.

The following equations were used to calculate pigment content (expressed as mg ml^{-1}):

[Chlorophyll *a*] = $(12.7*E_{\lambda 664.5}) / 1000$ (Inskeep & Bloom 1985)

[Chlorophyll *c*] = $(E_{\lambda 631} - E_{\lambda 582}) - (0.297 \times E_{\lambda 664.5}) / 61.8$ (Seely et al. 1972)

where E_x = the measured absorption coefficients at wavelength of *x* nm.

Pigment concentrations were then converted to mg g^{-1} wet weight.

RNA and DNA determination

Absolute cellular concentrations of RNA and DNA (and RNA:DNA ratios) were used to determine the proportion of protein synthesis machinery per cell as a proxy for "growth potential" of thalli. RNA and DNA content were determined by extraction from a single tissue sample. Frozen samples stored in RNAlater were thawed, patted dry, and a 1-5 mg (wet weight) subsample was weighed and homogenised with a drill pestle in a solution containing 500 µL of urea (4 M), sodium dodecyl sulphate (SDS, 1%), trisodium citrate (1 mM), sodium chloride (0.2 M) and 5 µL of Proteinase K (A.R. Bridle *unpub. data*). To ensure complete cell lysis, digestion of RNAses by Proteinase K, and stabilisation of nucleic acids, the homogenised solution was incubated at 37 °C for 10 minutes before being placed on ice. 750 µL of ammonium acetate (7.5 M) was added to the cooled solution, vortexed and centrifuged for 5 minutes at 14,000 RCF to precipitate impurities (e.g. chlorophyll, phenolic compounds, salts, detergents in urea/SDS, etc.). The resulting supernatant, containing total nucleic acid (tNA), was decanted into a 1.5 ml tube and precipitants discarded. 700 µL of isopropanol was added to the supernatant and tNA was pelletised by gently inverting the tube 40 times before centrifuging for 10 minutes at 14,00 RCF. The tNA pellet was washed twice in a 75% ethanol (EtOH) solution, resuspended in 200 µl molecular grade H_2O and split into two 100 µL aliquots.

A solution of 20 µL 10x DNase I reaction buffer (New England Biolabs – B0303S), 5µL DNase I (New England Biolabs - M0303L) and 80 µL molecular grade water was added to one aliquot of tNA suspension for total DNA digestion, and 5 µL of RNAse A (Sigma

Aldrich - R6148) with 100 µL of molecular grade water was added to the other aliquot to digest total RNA. Both tubes were incubated at 37 °C for 20 minutes to facilitate digestion (as per supplier's recommendations) then stabilised on ice. Impurities were further precipitated by vortexing in 400 µL of a urea/SDS buffer followed by vortexing and centrifuging with 200 µL of ammonium acetate (7.5 M). Isopropanol was added to the two aliquots of supernatant and total RNA and DNA precipitated as per the previous step. RNA and DNA pellets were washed twice in 75% EtOH and resuspended into 100 µl of molecular grade water (RNA) or EB buffer (DNA). Final concentrations of RNA and DNA were quantified by fluorescence assays performed using a Quibit assay probe and fluorometer. Nucleic acid concentrations were expressed as total RNA and DNA (µg.g wet weight^{-1}) which were used to calculate RNA:DNA ratios. Within-sample variability from a subset of duplicate samples (>20% of total samples extracted) gave the variability for *Ecklonia*, *Phyllospora* and *Macrocystis* (respectively) as follows: total RNA: ± 15.1, 14.8 and 6.6%; total DNA: ± 17.5, 17.0 and 11.9%; RNA:DNA: ± 20.1, 14.2 and 10.8% (mean percentage standard error of duplicate samples).

4.3.3 Environmental measurements

Temperature and nutrients

Water column characteristics at each location were sampled from three randomly selected GPS points within 100 m of the seaweed sampling site each time biological measurements were taken. Water temperature and salinity profiles were recorded with a YSI 6600V2 sonde (YSI Inc., Yellow Springs, OH, USA). Water temperature data was also collected by a nearby offshore monitoring buoy at Maria Island, Tasmania (42.597 °S, 148.233° E; Integrated Marine Observing System 2013). At each site, water was collected for chemical analysis of total dissolved phosphorus, nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_3) from depths of 2 m and 15 m (to identify potential heterogeneity in nutrients throughout the water column) using a Niskin bottle. Duplicate samples were taken at each depth/site combination and immediately filtered through 0.4 µm polycarbonate filters into plastic HDPE vials. Samples were held on ice in darkness for transport before being frozen at -20 °C within 6 hours, where they were kept for <1 month before flow injection analysis (Analytical Services Tasmania).

Irradiance

Photosynthetically active radiation (PAR, 400-700 nm) was measured *in situ* over the duration of seaweed monitoring using Odyssey data-loggers (Dataflow Systems, New Zealand). Loggers were deployed at depths of 10 m and 30 m at each location in open areas (no canopy) in the proximity of sampled seaweed. Each logger was held in a purpose-built rack mounted to a vertical galvanised rod sunken into a concrete-filled tyre. Each rack also held a custom-built mechanical wiper system which brushed the sensor window of the logger at 30 minute intervals to prevent sedimentation and biofouling. As loggers at NSW sites could only be checked (and replaced) at 6 monthly intervals, extensive fouling by barnacle growth occurred and data was only available over winter/spring each year. Short discontinuities also occurred in the stream of logged data from Tasmania when loggers were removed and replaced with fresh units. All loggers were calibrated underwater against a manufacturer-calibrated quantum sensor (Li-Cor 250m, Li-Cor Inc., Lincoln, NE, USA). Units were set to log cumulative PAR data over a period of 15 minutes, and total daily photon exposure (mol m^{-2} d^{-1}) was calculated. These values were converted to two day averages to smooth daily variability and plotted against time, along with total daily solar exposure (kW m^{-2}) obtained from adjacent terrestrial monitoring stations (Bureau of Meteorology). The equation described by Kirk (2003) was used to calculate the diffuse vertical attenuation coefficient of downwards irradiance (K_d) according to the formula:

$$K_d = \frac{1}{Z_2 - Z_1} \times \ln\left(\frac{E_{d(Z_1)}}{E_{d(Z_2)}}\right) \qquad (1)$$

where $E_{d(Z1)}$ and $E_{d(Z2)}$ = irradiances at depths Z_1 and Z_2, respectively. The euphotic zone depth, $Z_{1\%}$, was also calculated from the above equation. K_d and $Z_{1\%}$ were only determined for Tasmania due to the large discontinuities in shallow (10 m) irradiance data from NSW.

4.3.4 Statistical analysis

All measured ecophysiological characteristics ($rETR_{max}$, E_k, F_v/F_m, % C, % N, C:N, $\delta^{13}C$, $\delta^{15}N$, absolute RNA and DNA, RNA:DNA ratios, chlorophyll *a* and *c*, fucoxanthin) of NSW and Tasmanian seaweeds were plotted against time. Analysis of Variance (ANOVA) was conducted on all response variables for *Ecklonia* and *Phyllospora* to determine the significance of differences in seasonal physiological patterns of high- and low-latitude

seaweeds and (in the case of *Ecklonia*) deep and shallow individuals. A four-way factorial design was used for *Ecklonia*, with the factors 'year' (random), 'depth' (random), 'season' and 'location' (fixed). This was reduced to a three-way design for *Phyllospora*, with the omission of the factor 'depth'. The factor 'year' was defined as the time following the first sampling occasion. As sampling in NSW was conducted only every 6 months (summer/winter), data from the Tasmanian site were also reduced to 6 monthly summer/winter sampling (total of 4 occasions) for the factor of 'season'. The timing of seasonal sampling did not always match perfectly between NSW and Tasmania, so summer and winter seasons were grouped between the two locations based on similarity of environmental conditions (see Table 4.1). For all ANOVA, data were checked for conformity to the assumptions of homoscedasticity and normality of residuals. Where heteroscedastic, the transformations to stabilise variances were determined by the relationship between SDs and means of groups (Draper & Smith 1981). Where factors had a significant effect on a dependent variable, *a posteriori* multiple comparisons were conducted using Tukey's HSD tests. No formal statistical analysis was conducted for *Macrocystis*, as this species was only sampled at a single depth and location and thus temporal (yearly and/or seasonal) patterns could be adequately described graphically.

Multivariate Analysis of Variance (MANOVA) was conducted on normalised response variables for both *Ecklonia* and *Phyllospora* to identify potential differences in 'overall physiological state' across the various seasons, depths and locations. Separate MANOVAs were also run for photosynthetic parameters, pigments, tissue chemistry, and nucleic acids to isolate whether particular 'groups' of ecophysiological measurements were driving overall significance of effects. The statistical package 'R' (v 3.0.0) was used for all ANOVA and MANOVA analyses.

Distance-based Linear Models (distLMs) were performed on each species to analyse which subset of environmental variables best predicted the multivariate biological signal ($rETR_{max}$, E_k, F_v/F_m, % C, % N, C:N, $\delta^{13}C$, $\delta^{15}N$, absolute RNA and DNA, RNA:DNA ratios) at each depth (for *Ecklonia*), location (for *Ecklonia* and *Phyllospora*) and sampling occasion (all species). Eight predictor variables were entered into the models: water temperature, NO_3^-, NO_2^-, NH_3, dissolved reactive phosphorus, the ratio of dissolved inorganic nitrogen (DIN, calculated as $[NO_3^-] + [NO_2^-] + [NH_3]$) to dissolved inorganic phosphorus (DIP), surface irradiance, and *in situ* irradiance. For *Ecklonia*, *in situ* irradiance was derived from PAR

loggers (no canopy) at 10 m and 30 m; for *Phyllospora* and *Macrocystis*, only surface irradiance was used as *in situ* irradiance data was not available from beneath seaweed canopies at ~6 m and ~1 m depth (respectively). Chlorophyll *a*, *c* and fucoxanthin were omitted because pigments were not sampled on all occasions. For NSW *Ecklonia*, March 2011 and February 2012 occasions were omitted from the model because *in situ* irradiance was not available at this time. For *Macrocystis*, DIN:DIP was excluded as a predictor variable due to the limited number of biological measurements relative to environmental variables. Biological data were first normalised and a Euclidean distance resemblance matrix generated, and all environmental variables were tested for collinearity (Draftsman plot and Spearman correlation matrix). No pair of variables was highly linearly correlated (indicated by $r > 0.95$) so all variables were retained for possible inclusion in the model. distLMs were constructed using the step-wise and best selection procedure with 999 permutations (and models compared for congruence), and the Akaike's Information Criterion (AIC) was used to fit the best explanatory environmental variables in the models. Results were visualised with a distance-based redundancy analysis (dbRDA). PRIMER-E software (v 6.1.12) was used for all multivariate distLM and dbRDA analyses.

4.4 Results

4.4.1 Tissue chemistry and isotopic signatures

Ecklonia

In general, *Ecklonia* had similar levels of % C and % N between depths within each location. However, unusually high % N in December 2010 of deep Tasmanian thalli, and divergence in % N of deep and shallow NSW thalli in March 2011 resulted in significant year × season × depth, and year × season × location interactions for % N (Table 4.2A; Fig. 4.1B). In general, Tasmanian *Ecklonia* exhibited a decrease in % N over summer, while NSW seaweeds showed no clear seasonality in either % C or % N (Fig. 4.1A,B). Deep *Ecklonia* consistently had lower C:N ratios than shallow thalli at both locations, while Tasmanian *Ecklonia* generally had lower C:N ratios than NSW individuals (Fig. 4.1C), although inter-annual variability in seasonal patterns of C:N ratios resulted in significant year × season × depth, and year × season × location interactions (Table 4.2A). NSW *Ecklonia* (both depths), and shallow Tasmanian *Ecklonia* were N-limited for much of the year (except during winter in Tasmania), indicated by low values of thallus N and/or high C:N ratios (>15, N-limitation threshold for

seaweed; Lapointe 1987, Frederiksen & Rueness 1989, Rico & Fernández 1996). Deep Tasmanian *Ecklonia* consistently maintained the highest thallus % N and lowest C:N ratio, indicating a lack of N limitation in these seaweeds (Fig. 4.1B & C).

The least negative values of $\delta^{13}C$ (~-16‰) occurred in shallow Tasmanian *Ecklonia* and were maintained throughout most of the year but decreased over winter (Fig. 4.1D). Shallow NSW *Ecklonia* maintained lower and more seasonally consistent values of $\delta^{13}C$, while deep NSW thalli demonstrated a pattern in $\delta^{13}C$ similar to Tasmanian seaweeds (least negative in summer, most negative in winter) but with overall more negative values. Deep Tasmanian *Ecklonia* maintained the most negative values of $\delta^{13}C$, increasing only during summer but remaining consistently below ~-21‰ (Fig. 4.1D). Patterns of $\delta^{15}N$ were similar between deep and shallow *Ecklonia* in Tasmania, with slight increases from ~7.5 in summer to ~9.0 in winter (Fig. 4.1E). NSW *Ecklonia* maintained consistently lower values of $\delta^{15}N$ (~4.6 – 6.6) than their Tasmanian counterparts, with no clear seasonal or depth pattern (reflected as significant year × season × depth × location interaction, $p < 0.01$; Table 4.2A).

Phyllospora

No clear seasonal pattern was evident in % C, % N or C:N ratios in *Phyllospora* from either origin (although C:N of Tasmanian thalli was consistently highest in spring/summer; Fig. 4.1A-C). As with *Ecklonia*, NSW *Phyllospora* were generally more N-limited (higher C:N ratios) than Tasmanian thalli except during summer 2012 when % C (and thus C:N) of NSW thalli was low, although *Phyllospora* thalli at both locations were N-limited throughout the study (Fig. 4.1A-C). Values of $\delta^{13}C$ showed no clear seasonal patterns and were similar in both locations (Fig. 4.1D), as was the fraction of the heavy N isotope $\delta^{15}N$ (no significant location effect; Table 4.2B, Fig. 4.1E).

Macrocystis

Of all three species, *Macrocystis* exhibited the strongest seasonality in % C, % N and C:N ratios (Fig. 4.1). Values of % C decreased in late autumn (May-June), while % N was low during spring-summer (November-February), resulting in N limitation for approximately half the year (C:N > 15 over spring-summer; Fig. 4.1A-C). Values of $\delta^{13}C$ were similar to Tasmanian *Phyllospora* and shallow Tasmanian *Ecklonia*, and showed little seasonal variation (Fig. 4.1D), while $\delta^{15}N$ values were also similar to other Tasmanian species (~6-9‰) and varied seasonally, reaching maximum values in late spring (Fig. 4.1E).

Table 4.2 Factorial ANOVA testing the effect of location (NSW *vs.* Tasmania), year (2 levels), and depth (10 m *vs.* 30 m for *Ecklonia* only) on seasonal (winter *vs.* summer) patterns in photosynthetic characteristics, pigments, tissue chemistry and nucleic acid composition of **(A)** *Ecklonia radiata*; and **(B)** *Phyllospora comosa* measured *in situ.* Abbreviations: chlorophyll *a* (chl *a*), chlorophyll *c* (chl *c*), fucoxanthin (fucox). The factor 'year' is excluded for pigments as these were not measured over the full two years. *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

A *Ecklonia*		Pigments				Tissue chemistry					Photosynthetic characteristics			Nucleic acids		
Factor	*df*	Chl *a*	Chl *c*	Fucox	*df*	% C	% N	C:N	$\delta^{13}C$	$\delta^{15}N$	$rETR_{max}$	E_k	F_v/F_m	RNA	DNA	RNA:DNA
Location (L)	1	***	**	*	1	***	***	***	n.s.	***	***	***	***	***	***	***
Depth (D)	1	***	*	***	1	n.s.	***	***	***	*	***	***	n.s.	n.s.	***	***
Season (S)	1	***	***	***	1	***	***	***	***	*	***	***	**	n.s.	n.s.	***
Year (Y)	-	-	-	-	1	***	***	***	*	***	n.s.	n.s.	***	**	n.s.	n.s.
L x D	1	***	**	***	1	n.s.	n.s.	n.s.	***	n.s.	***	n.s.	***	n.s.	***	***
L x S	1	***	n.s.	***	1	***	n.s.	*	n.s.	*	n.s.	***	***	n.s.	**	***
L x Y	-	-	-	-	1	***	n.s.	n.s.	n.s.	n.s.	***	**	***	n.s.	n.s.	n.s.
D x S	1	n.s.	**	n.s.	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	***	**
D x Y	-	-	-	-	1	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
S x Y	-	-	-	-	1	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L x D x S	1	**	n.s.	*	1	n.s.	n.s.	n.s.	***	n.s.	**	*	***	n.s.	n.s.	*
L x D x Y	-	-	-	-	1	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	n.s.	n.s.	n.s.	n.s.
L x S x Y	-	-	-	-	1	n.s.	***	***	n.s.	n.s.	***	***	n.s.	n.s.	n.s.	n.s.
D x S x Y	-	-	-	-	1	n.s.	***	*	n.s.	n.s.	*	***	n.s.	n.s.	n.s.	n.s.
L x D x S x Y	-	-	-	-	1	n.s.	n.s.	n.s.	n.s.	**	***	***	n.s.	n.s.	n.s.	n.s.
residuals	72				144											

Table 4.2 (cont.)

B *Phyllospora*		Pigments				Tissue chemistry					Photosynthetic characteristics			Nucleic acids		
Factor	*df*	Chl *a*	Chl *c*	Fucox	*df*	% C	% N	C:N	$\delta^{13}C$	$\delta^{15}N$	$rETR_{max}$	E_k	F_v/F_m	RNA	DNA	RNA:DNA
Location (L)	1	***	*	***	1	***	***	**	*	n.s.	***	***	***	***	***	n.s.
Season (S)	1	***	***	***	1	***	*	n.s.	n.s.	n.s.	***	***	**	n.s.	n.s.	n.s.
Year (Y)	-	-	-	-	1	***	***	n.s.	n.s.	n.s.	*	**	n.s.	n.s.	***	**
L x S	1	***	**	***	1	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.
L x Y	-	-	-	-	1	***	**	***	***	n.s.	***	***	**	n.s.	***	n.s.
S x Y	-	-	-	-	1	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.
L x S x Y	-	-	-	-	1	n.s.	***	***	n.s.	n.s.	**	*	*	n.s.	n.s.	n.s.
residuals	36				72											

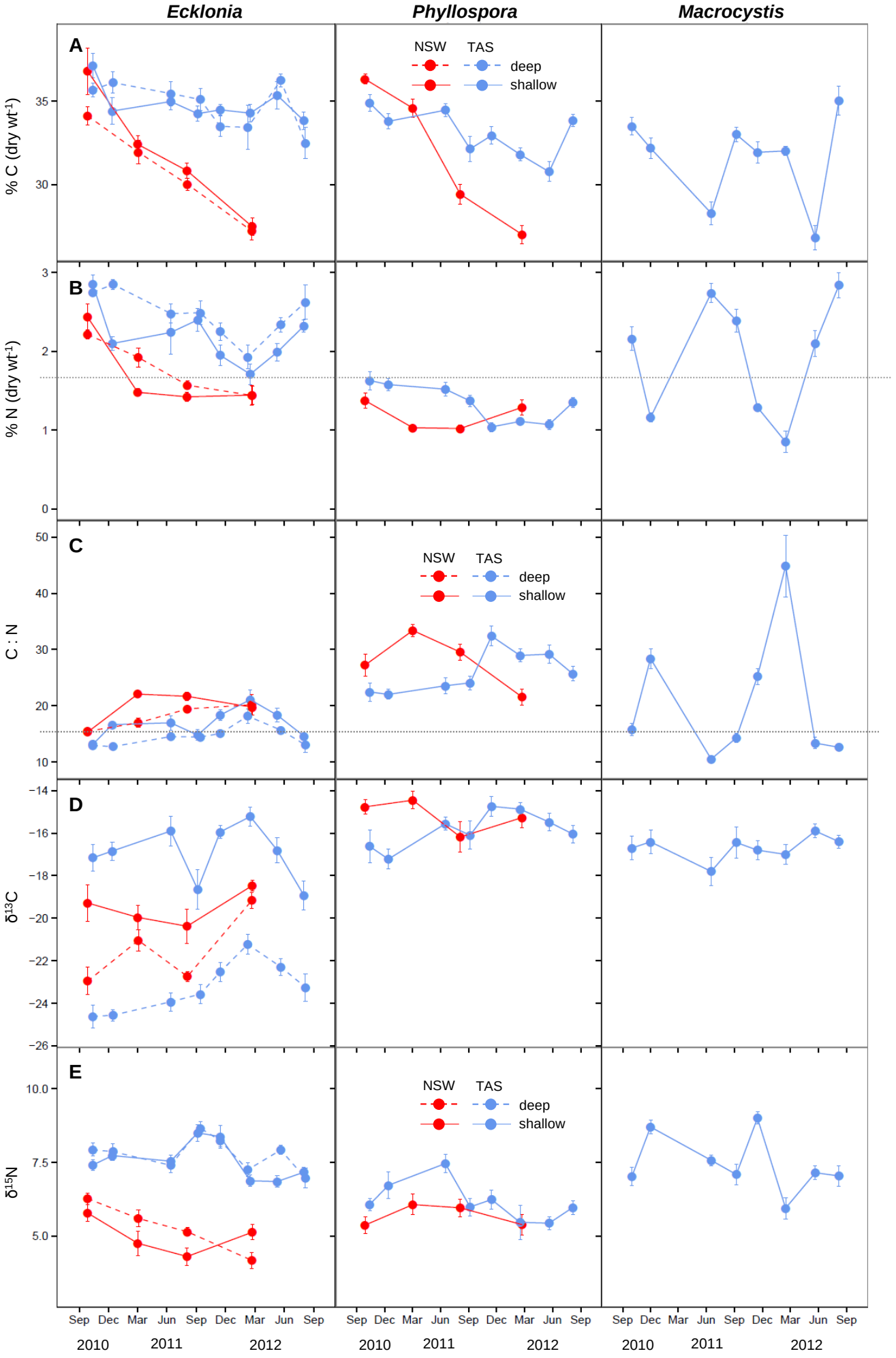

Ecklonia
Phyllospora
Macrocystis
A
B
C
D
E
NSW
TAS
deep
shallow
% C (dry wt-1)
% N (dry wt-1)
C : N
δ13C
δ15N
Sep Dec Mar Jun Sep Dec Mar Jun Sep
2010
2011
2012

Figure 4.1 Variation in chemical and isotopic characteristics of adult *Ecklonia radiata* and *Phyllospora comosa* in NSW and Tasmania, and *Macrocystis pyrifera* (Tasmania only), from September 2010 to August 2012 (means ± SE, n = 10). *Ecklonia* was sampled at two depths (10 m, solid lines; 30 m, dashed lines). (**A-B**) C and N thallus content (% dry weight), (**C**) C:N ratio, (**D-E**) $\delta^{13}C$ and $\delta^{15}N$ values. Dotted lines in (**B**) and (**C**) indicate (respectively) the critical N value (1.7%) required to maintain maximum growth, and the N-limitation threshold (C:N = 15).

4.4.2 Photosynthetic characteristics and pigment content

Ecklonia

Seasonal patterns in photosynthetic characteristics of *Ecklonia* varied between deep and shallow thalli and between locations (significant location × season × depth interaction, $p < 0.05$ for all parameters), while $rETR_{max}$ and E_k were also influenced by the sampling year (reflected as a significant location × season × depth × year interaction, $p < 0.0001$ for both; Table 4.2A). For shallow thalli, $rETR_{max}$, E_k and F_v/F_m were highest in NSW individuals, peaking in summer and decreasing over winter when water temperature and irradiance were low. In contrast, shallow Tasmanian thalli demonstrated significant declines in all PAM-measured photosynthetic characteristics over late summer, which didn't increase again until spring (Table 4.2A; Fig. 4.2). While deep NSW *Ecklonia* followed a similar pattern of photosynthetic characteristics to shallow NSW thalli (albeit with lower values of $rETR_{max}$ and E_k) from 2010-2011, these photosynthetic parameters were anomalously low in summer 2012 (Fig. 4.2A, B). Values of $rETR_{max}$ and E_k of deep Tasmanian *Ecklonia* showed no clear seasonal patterns (although interpretation may be partially complicated by to the absence of a true "summer" sampling in 2010-2011). Both $rETR_{max}$ and E_k were low in Tasmanian *Ecklonia* in late spring (November/December, both years) which corresponded with increased light attenuation (Fig. 4.6C), but the decline in these parameters over late summer/autumn was not as evident as for shallow Tasmanian thalli. Importantly, whereas NSW *Ecklonia* and deep Tasmanian thalli maintained similar and seasonally consistent values of F_v/F_m (~0.78-0.80), shallow *Ecklonia* in Tasmania had significantly lower values of F_v/F_m (~0.68-0.72) during summer when temperature and irradiance were high (Figs 4.2C & 4.6).

Deep and shallow *Ecklonia* at each location showed similar seasonal patterns in their concentrations of photosynthetic pigments, but this varied between locations (Table 4.2A; Fig. 4.3). Tasmanian seaweeds had greater pigment concentrations than NSW individuals for much of the year, except during summer when pigments converged to similarly low

concentrations in seaweeds from both locations. All thalli increased their pigment content over winter (corresponding with reduced temperature and PAR; Fig. 4.6), but this was particularly pronounced in Tasmanian *Ecklonia* which exhibited a 3 to 30-fold increase in pigment concentration between summer and winter (Fig. 4.3). In general, *Ecklonia* at depth in NSW contained more pigments shallower thalli at the same location, while deep and shallow thalli in Tasmania shared a similar pigment content year-round except during winter when shallow individuals displayed a greater concentration of chlorophyll *a*, *c* and fucoxanthin.

Phyllospora

In contrast to *Ecklonia*, values of $rETR_{max}$ and E_k increased over summer in *Phyllospora* at both locations (although it was difficult to determine whether this was temporally consistent due to the absence of a true summer sampling in 2010-11). However, the magnitude of seasonal fluctuations (i.e. difference between winter and summer values) was much greater in NSW (Fig. 4.2A & B). Significant year × season × location interactions for these parameters was likely caused by a significant year × season effect due to the missed summer sampling in Tasmania (2011), rather than different seasonal patterns between locations (Table 4.2B). Despite that Tasmanian sampling only adequately captured a single summer, values of F_v/F_m declined from ~0.77 to 0.69 over 2011-12 summer in Tasmanian thalli whereas F_v/F_m remained high (~0.77) and seasonally consistent in NSW individuals (Fig. 4.2C). Seasonal patterns, spatial trends, and absolute concentrations of photosynthetic pigments were similar to those documented for *Ecklonia*, with Tasmanian *Phyllospora* containing more pigments than NSW thalli except during summer when pigment concentrations converged to similarly low values at both locations (Fig. 4.3). As with *Ecklonia*, chlorophyll *c* was the only pigment to change significantly between seasons in NSW thalli, while Tasmanian seaweeds demonstrated strong seasonal patterns in all measured pigments.

Macrocystis

The between-plant variability in $rETR_{max}$ and E_k was much greater in *Macrocystis* than for the other two species (particularly in E_k; Fig. 4.2B) which made robust interpretation of seasonal patterns difficult. However, F_v/F_m displayed a strong seasonal patterns of decreasing by ~30% from ~0.77 in autumn/winter to ~0.58 over spring/summer (Fig. 4.2C). Seasonal fluctuations and absolute concentrations of all measured pigments were also similar to those documented for *Ecklonia* and *Phyllospora*, although pigment concentrations more closely tracked patterns of irradiance (i.e. less of a "lag" in pigment synthesis following a decrease in subsurface PAR; Figs 4.3 & 4.6).

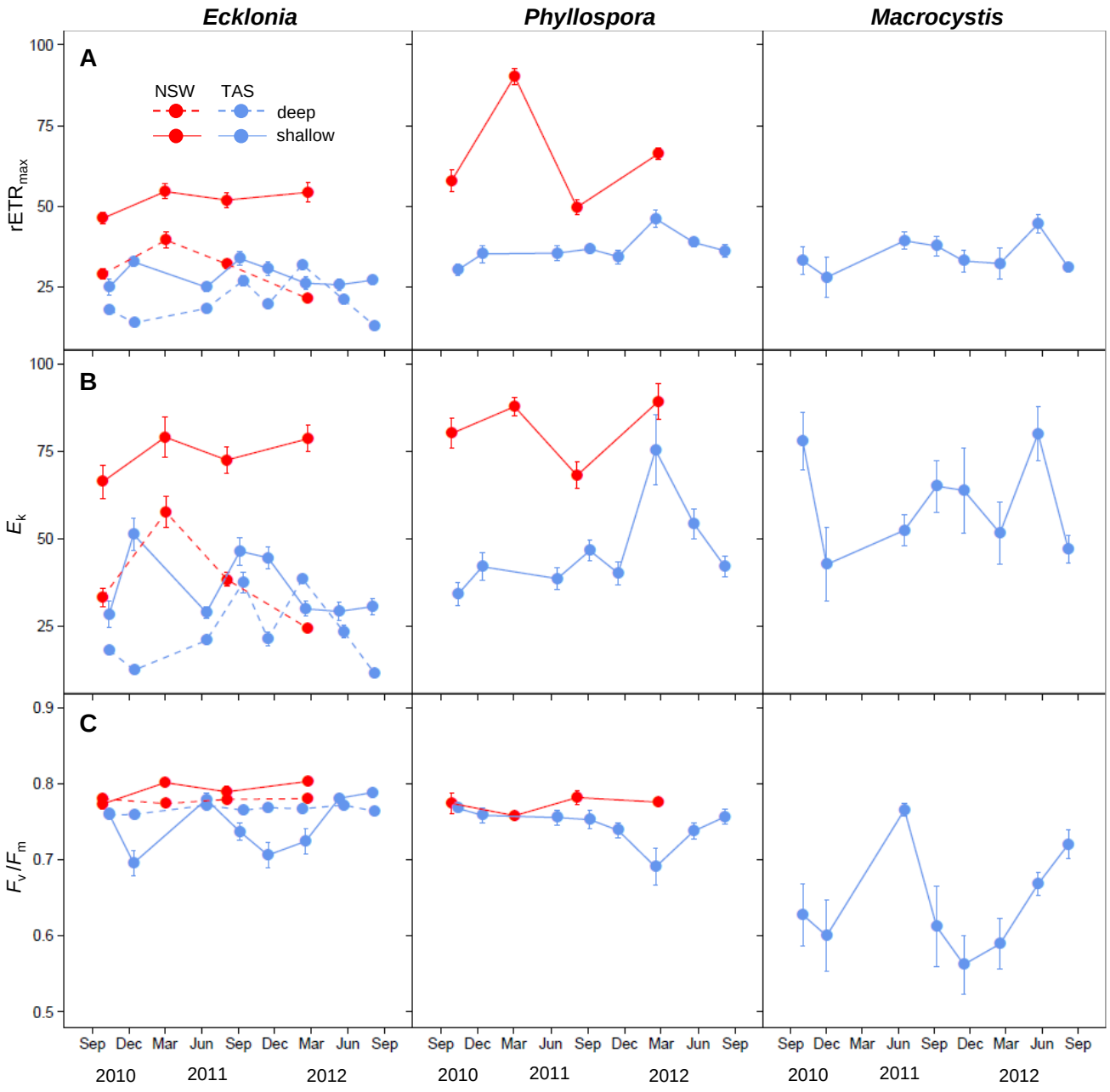


Figure 4.2 Variation in photosynthetic characteristics of adult *Ecklonia radiata* and *Phyllospora comosa* in NSW and Tasmania, and *Macrocystis pyrifera* (Tasmania only), from September 2010 to August 2012 (means ± SE, n = 10). *Ecklonia* was sampled at two depths (10 m, solid lines; 30 m, dashed lines). (**A**) Maximum relative electron transport rate, $rETR_{max}$; (**B**) saturating irradiance, E_k; (**C**) maximum quantum yield, F_v/F_m.

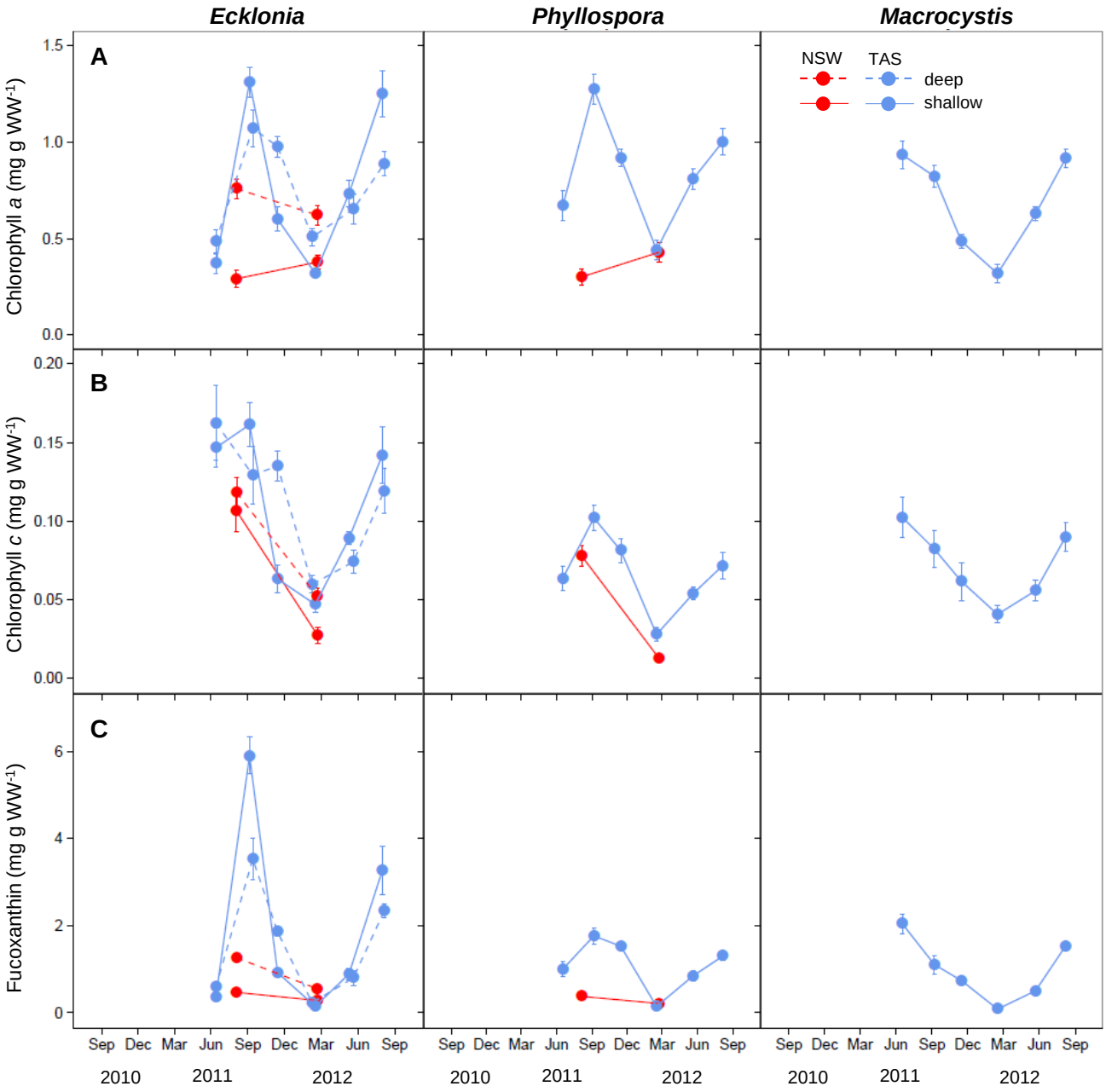


Figure 4.3 Variation in the concentration of photosynthetic pigments of adult *Ecklonia radiata* and *Phyllospora comosa* in NSW and Tasmania, and *Macrocystis pyrifera* (Tasmania only), from June 2011 to August 2012 (means ± SE, n = 10). *Ecklonia* was sampled at two depths (10 m, solid lines; 30 m, dashed lines). (**A**) Chlorophyll *a*; (**B**) chlorophyll *c*; (**C**) fucoxanthin.

4.4.3 Nucleic acids

Ecklonia

While deep and shallow *Ecklonia* had similar absolute concentrations of RNA (no significant depth effect; Table 4.2A, Fig. 4.4A), deep *Ecklonia* generally maintained higher concentrations of DNA than shallower thalli at the same location (although deep and shallow thalli in Tasmania shared similar concentrations of DNA during winter; Fig. 4.4B). Tasmanian *Ecklonia* had higher concentrations of both RNA and DNA than NSW individuals at the same depth, although for DNA the effect of location interacted with depth and season (Table 4.2A). No strong seasonal patterns in RNA concentration were detected at either location (Table 4.2A), but DNA concentrations in shallow NSW *Ecklonia* decreased over summer to the extent that RNA:DNA ratios more than doubled (from ~10 to 20-25), while Tasmanian *Ecklonia* (both depths) and deep NSW thalli maintained a consistent RNA:DNA ratio of ~9 across seasons and years, resulting in in a significant location × season × depth interaction (Table 4.2; Fig. 4.4C).

Phyllospora

The RNA content of Tasmanian *Phyllospora* showed a similar (but not statistically significant) seasonal pattern to *Ecklonia* at the same location (Table 4.2B; Fig. 4.4A). NSW thalli also showed no clear seasonal patterns in RNA concentrations, and DNA concentrations were seasonally consistent at both locations (no significant season effect; Table 4.2B; Fig. 4.4A,B). As for *Ecklonia*, Tasmanian *Phyllospora* contained greater concentrations of both RNA and DNA relative to NSW individuals, and *Phyllospora* from both locations had lower concentrations of nucleic acids (particularly DNA) than *Ecklonia* (Fig. 4.4). Ratios of RNA:DNA were similar between locations, and followed no clear seasonal patterns (no significant location or season effect; Table 4.2B; Fig. 4.4C)

Macrocystis

Peaks in concentrations of RNA and DNA occurred in *Macrocystis* in late winter/early spring, with decreases in the concentration of both nucleic acids occurring over summer (Fig. 4.4). As DNA concentration (not RNA) was the primary driver of changes in RNA:DNA ratios in this species, RNA:DNA ratios were relatively consistent throughout most of the year, with a strong peak in RNA:DNA occurring over summer 2011-12 as the absolute concentration of DNA declined (summer 2010-11 not adequately sampled).

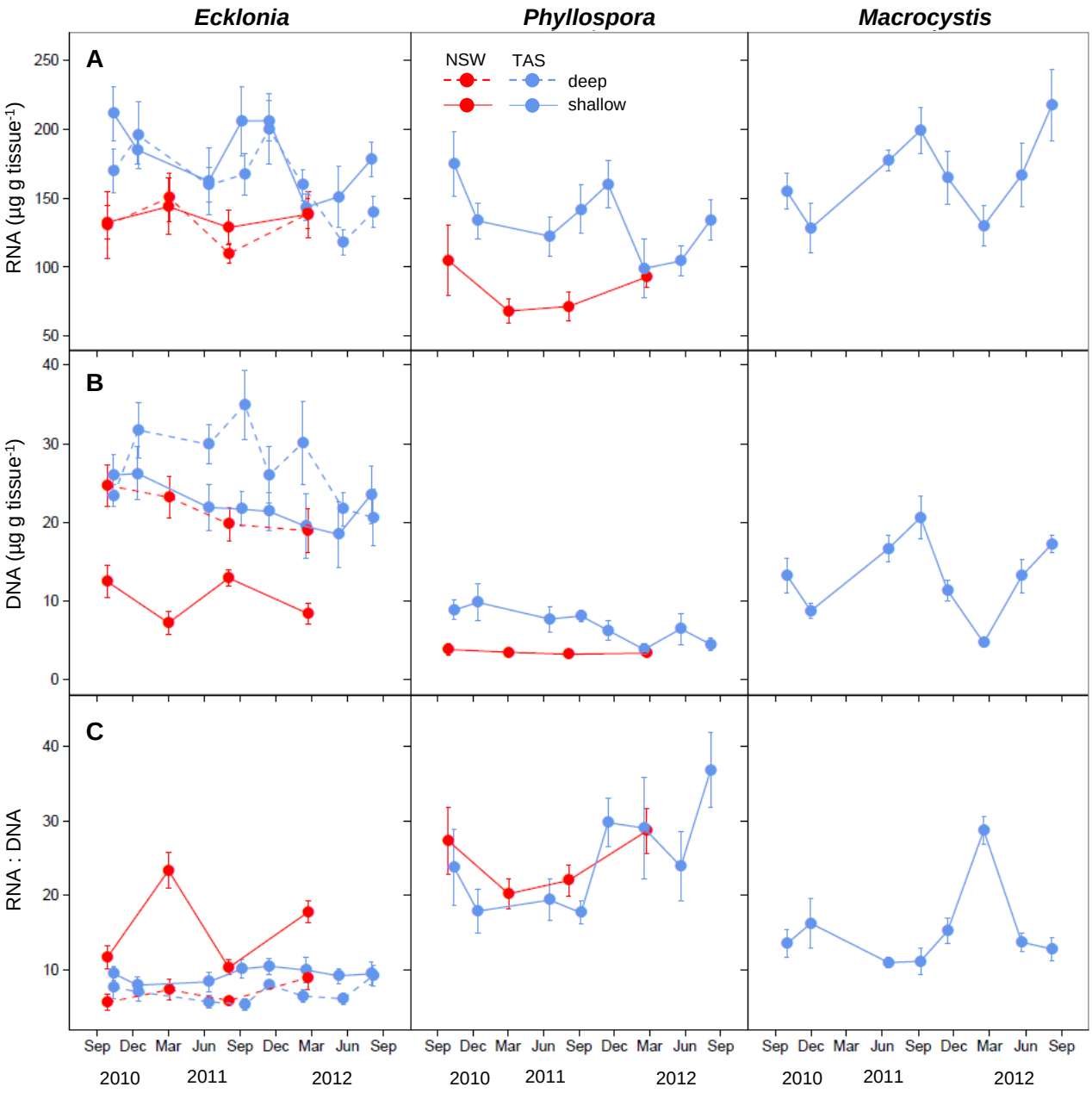


Figure 4.4 Variation in the nucleic acid content of adult *Ecklonia radiata* and *Phyllospora comosa* in NSW and Tasmania, and *Macrocystis pyrifera* (Tasmania only), from September 2010 to August 2012 (means ± SE, *n* = 10). *Ecklonia* was sampled at two depths (10 m, solid lines; 30 m, dashed lines). (**A**) Absolute RNA concentration; (**B**) absolute DNA concentration; (**C**) RNA : DNA ratio.

4.4.4 Multivariate analysis

Multivariate ANOVA (MANOVA) on all response variables collectively did not reveal any further insight to the individual univariate ANOVAs, with the highest order interaction (season × year × location × depth for *Ecklonia*, or season × year × location for *Phyllospora*) significant for both species ($F_{11,106} = 7.36$, $p < 0.0001$ and $F_{11,54} = 4.98$, $p < 0.0001$, respectively).

4.4.5 Physical environment

Seasonal patterns in dissolved nutrients were similar between depths and locations, with the exception of NO_3^- measured at 15 m in NSW over summer 2012 which was unseasonably high (Fig. 4.5) suggesting incursions of water from depth. In general, nutrients peaked in winter and were low over spring/summer (frequently at undetectably low levels), although the greater sampling frequency in Tasmania revealed that NH_3 and NO_2^- peaked slightly earlier (during late autumn).

PAR at 10 m depth was approximately an order of magnitude greater than at 30 m within the same location (Fig. 4.6B). While subsurface PAR data was not available in NSW over summer, surface irradiance data showed the Tasmanian site was subject to greater insolation than the NSW site over summer, but winter insolation was approximately 50% less than at the NSW site. Peaks in subsurface irradiance (January) generally occurred later than peaks in surface irradiance (December) due to seasonal fluctuations in light attenuation through the water column (K_d; Fig. 4.6C). Maximum water temperature and subsurface PAR occurred at a similar time, and NSW was consistently 4-6 °C warmer than Tasmania, with NSW winter temperatures similar to Tasmanian summer temperatures (Fig. 4.6A).

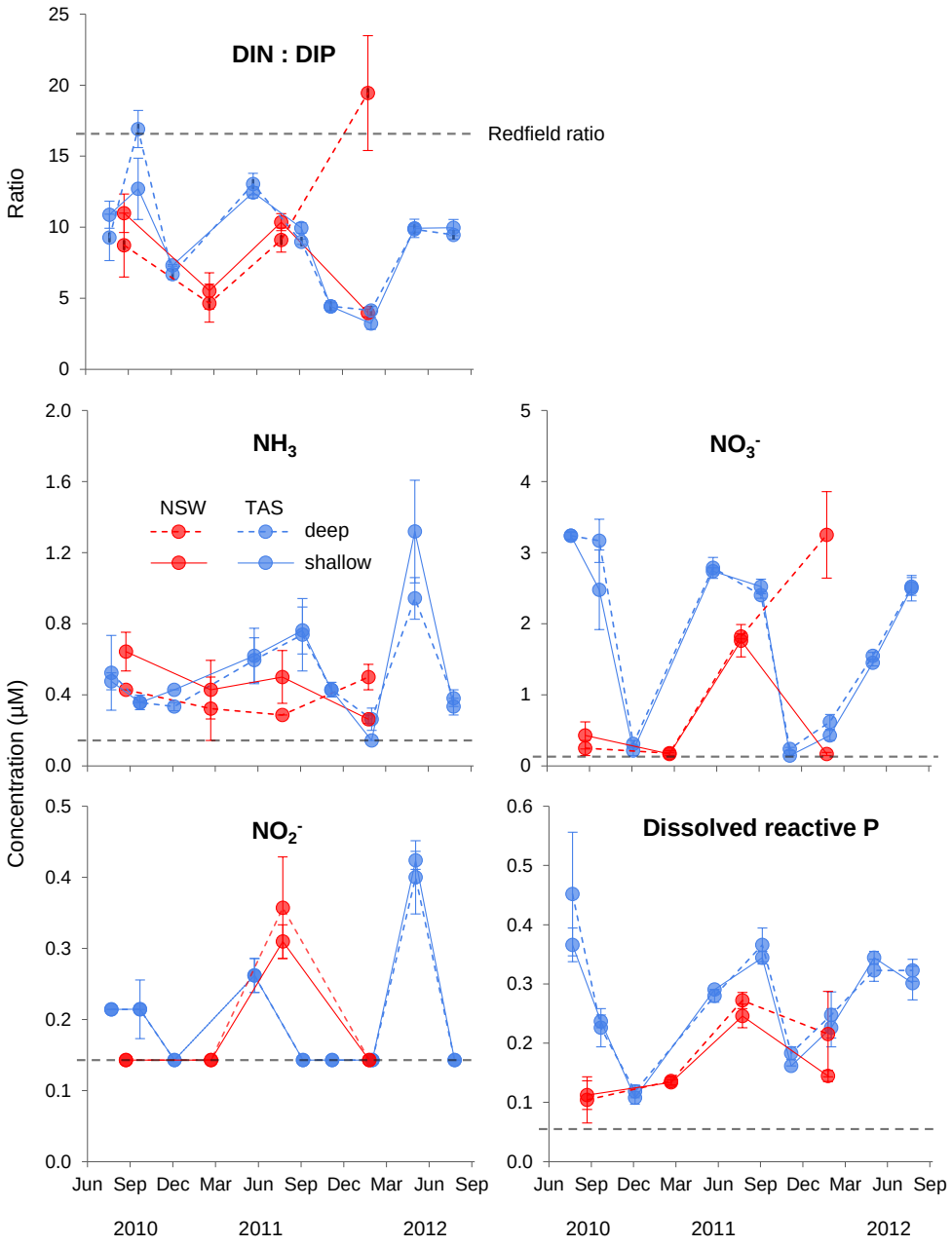


Figure 4.5 Variation in the dissolved nutrient composition of seawater at depths of 2 m and 15 m at NSW and Tasmanian sites between October 2010 and August 2012. Values are given as means across seaweed sampling sites within a location (n = 3 for NSW; n = 4 for Tasmania) SE. Dotted lines in the four bottom panels indicate the minimum detection threshold for each nutrient (0.143 µM for NH_3, NO_3^-, NO_2^-; 0.065 µM for dissolved reactive P). Dissolved inorganic nitrogen : phosphorus (DIN:DIP) indicates the sum of all measured N sources relative to total dissolved reactive P.

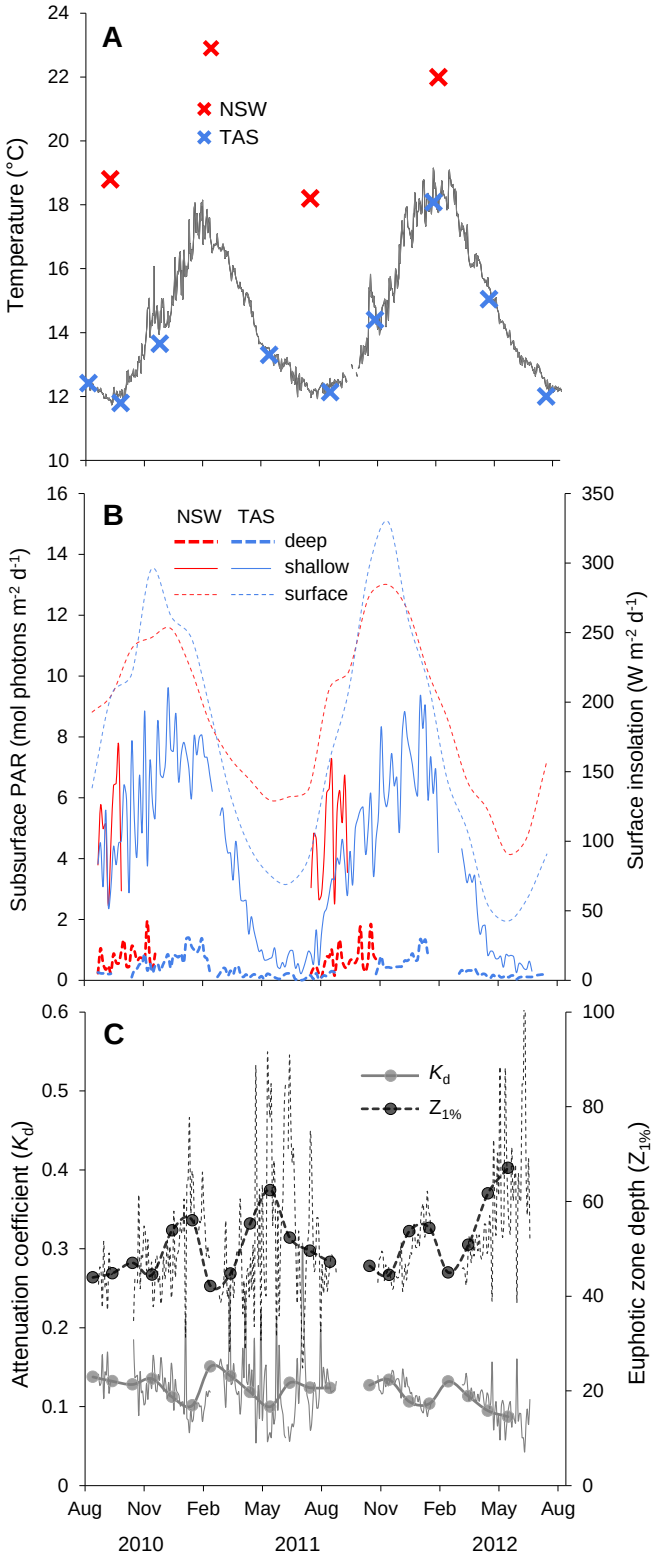


Figure 4.6 Seasonal and inter-annual values of **(A)** temperature and **(B)** irradiance at NSW and Tasmanian sites, and **(C)** light attenuation in the water column (Tasmania only), from August 2010-2012. The raw curve in **(A)** shows Tasmanian east coast temperature data from an offshore monitoring buoy; symbols (×) show *in situ* temperature measurements at NSW and Tasmania study sites and indicate the timing

of seaweed ecophysiological measurements. Raw values in **(B)** show daily subsurface photosynthetically active radiation (plotted as a 2-day average) at shallow (10 m; solid line) and deep (30 m; dotted line) monitoring stations. Smoothed curves (secondary axis) show monthly averages of total surface insolation (all wavelengths). Discontinuities in raw values indicate periods of PAR logger failure and/or replacement. Raw values in **(C)** show light attenuation (grey solid line, primary axis) and euphotic zone depth (black dashed line, secondary axis) in Tasmania (plotted as 2-day average), with smoothed curves showing monthly averages.

4.4.6 Relationship between patterns in seaweed ecophysiology and environmental characteristics

distLM analyses indicated that the environmental variables most strongly influencing patterns in seaweed physiology varied between species. For *Ecklonia*, marginal tests indicated that temperature ($p = 0.002$) and *in situ* irradiance ($p = 0.006$) were the only significant individual environmental predictors of the multivariate physiological signal across times, locations and depths, but the best fitted model (using 'best' and 'step-wise' techniques) included P and DIN:DIP as well as temperature and *in situ* irradiance. Combined, these four factors accounted for ~91% and ~51% of the fitted and total variation, respectively (Fig. 4.7A). The vectors of the environmental variables retained by the distLM procedure are displayed in dbRDA plots (Fig. 4.7) and indicate the importance of each variable in explaining the separation of the *Ecklonia* multivariate physiological signal over time, between locations and depths. The physiological signal of NSW *Ecklonia* was separated from that of Tasmanian *Ecklonia* predominantly by temperature (and P to a lesser extent), while the signal of deep and shallow *Ecklonia* separated predominantly by PAR. Deep and shallow *Ecklonia* displayed similar physiological signals (within a location) over autumn/winter, while the signal of shallow *Ecklonia* differed from that of deep *Ecklonia* over spring/summer and was more broadly scattered in multivariate space (Fig. 4.7A).

The multivariate physiological signal of *Phyllospora* separated across time and locations by temperature (100% and ~38% of fitted and total variation, best and step-wise), with Tasmanian samples clearly separating from NSW samples (which grouped tightly into winter *versus* summer samples; Fig. 4.7B). An exception to this pattern was in the signal of Tasmanian *Phyllospora* sampled in Tasmania during summer (February 2012) which correlated with temperature in a similar manner to NSW thalli assessed in 'winter' (September 2010 and August 2011).

Macrocystis multivariate physiology was best explained by a model including all environmental variables (~68% and ~64% of fitted and total variation, best and step-wise; Fig. 4.7C). The multivariate signal of thalli sampled during summer and spring was affected predominantly by temperature and surface irradiance (*in situ* PAR not available), whereas the physiological signal of thalli sampled during winter was more strongly influenced by nutrients (predominantly NO_2^- and NH_3).

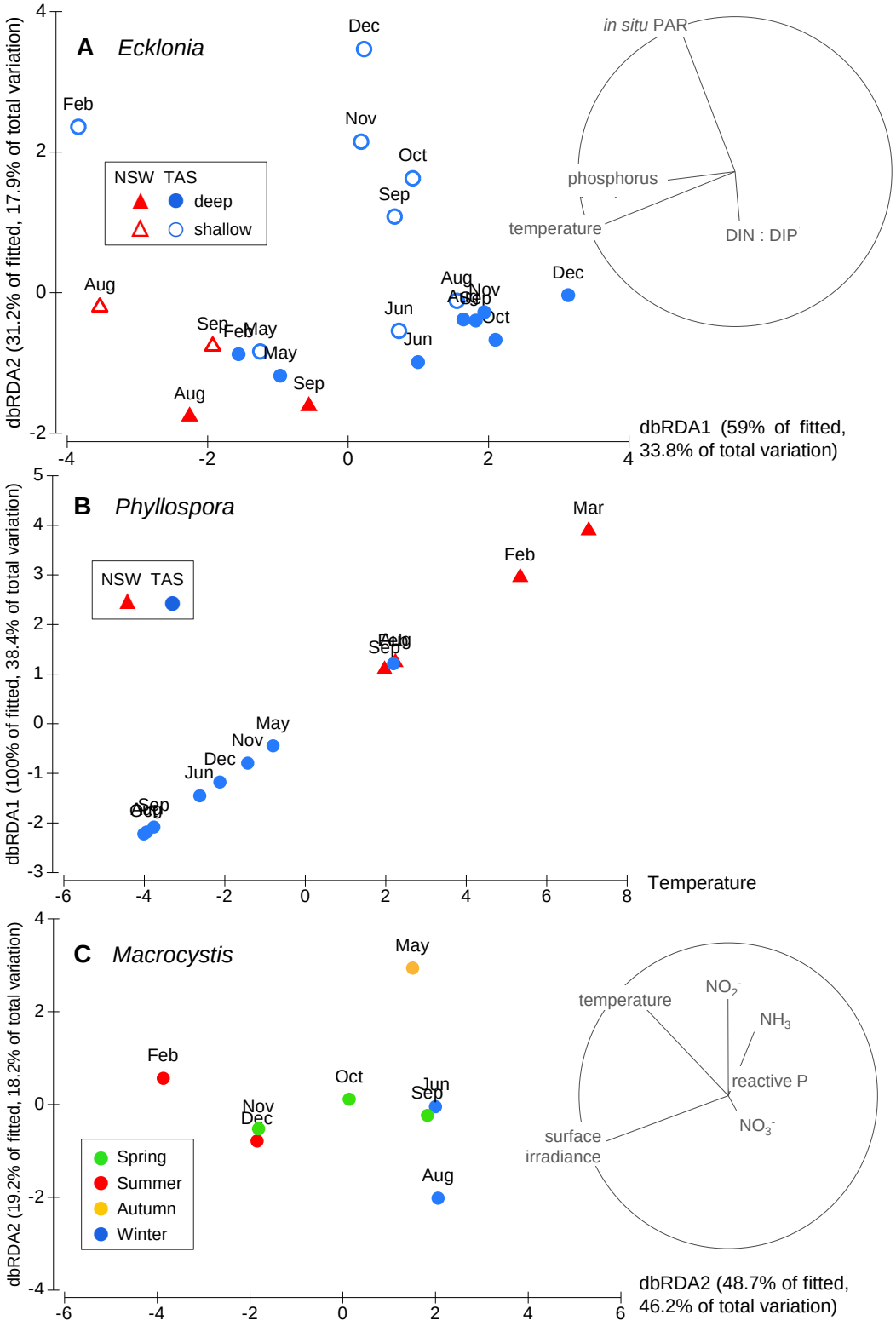


Figure 4.7 Distance-based redundancy analysis (dbRDA) plots illustrating the best-fit distLM model (indicated by stepwise analysis using Akaike's Information Criterion) of seasonal seaweed ecophysiological data and fitted environmental variables. (**A**) *Ecklonia radiata* (NSW and Tasmania, deep and shallow); (**B**) *Phyllospora comosa* (NSW and Tasmania); (**C**) *Macrocystis pyrifera* (Tasmania only). Vector plots in **(A)** and **(C)** show the strength and direction of the effects of environmental variables on the ordination plot (vector plot unnecessary for **(C)** as x-axis is a straight temperature axis).

4.5 Discussion

The *in situ* photosynthetic performance, tissue chemistry, pigment concentrations and nucleic acid content of *Ecklonia*, *Phyllospora* and *Macrocystis* was temporally variable, with many physiological characteristics following distinct seasonal patterns corresponding with variation in environmental conditions (particularly temperature but also light, with nutrients playing a less important role). However, the seasonal patterns of some characteristics also varied between sites and with depth. Ecophysiological interpretations were complex when considering individual variables in isolation; however the combined multivariate suite of characteristics showed that differences in the physiology of seaweeds in NSW and Tasmania (and, for *Ecklonia*, deep and shallow thalli) largely reflected latitudinal- and depth-related variation in abiotic factors. Temperature and irradiance were both important environmental regulators of *Ecklonia* physiology, while seasonal and latitudinal patterns in the physiology of *Phyllospora* thalli correlated predominantly with temperature. Importantly, the spatial variation in the measured characteristics (within a species) of both *Ecklonia* and *Phyllospora* indicates that thalli from higher latitudes are ecophysiologically different from their lower latitude conspecifics.

4.5.1 Seasonal and spatial patterns in environmental characteristics

Environmental conditions in NSW and Tasmania followed seasonal patterns typical of temperate waters (e.g. Sakanishi et al. 1989, Flores-Moya et al. 1995, Brown et al. 1997, Fairhead & Cheshire 2004a, Fairhead & Cheshire 2004b, Suthers et al. 2011), with temperature and irradiance peaking in summer (January/February) when nutrients were low, and vice versa for winter (July/August). Although surface irradiance reached maximum levels in early summer (December), water clarity at this time was low (presumably due to residual spring blooms) and thus subsurface PAR maxima did not occur until mid-late summer when nutrients were exhausted. Seaweeds in Tasmania were exposed to approximately double the magnitude of seasonal changes in daily PAR exposure between summer and winter relative to those in NSW (due to greater fluctuations in both solar intensity and day length), while temperatures were 4-6 °C lower in Tasmania than in NSW, although the actual ranges were similar (12-18 °C *vs.* 18-23 °C for Tasmania and NSW, respectively).

Seasonal variation in the nitrate content of seawater, from ~3.2 uM (Tasmania) in autumn-winter to 0.5 uM or less in spring-summer, was less than that reported for many other temperate regions (Chapman & Craigie 1977, Gerard 1982, Wheeler & Srivastava 1984), but is typical of the largely oligotrophic waters of southeastern Australia (Harris et al. 1987, Ridgway 2007). Nitrite and ammonium followed a similar seasonal pattern to NO_3^- such that the ratio of DIN:DIP was low over spring-summer and high in late autumn-winter. While both NH_3 and NO_2^- are also known to be utilised by seaweeds as an inorganic N source (Pedersen & Borum 1996, Ahn et al. 1998), the concentration of NO_3^- at both sites was nearly double that of NH_3 and NO_2^- combined (at the same site) at any given time of year, hence NO_3^- appears to be the primary source of N for seaweeds in this system (as has been reported elsewhere for open coastal systems, e.g. Gerard 1982, Wheeler & Srivastava 1984, Naldi & Viaroli 2002, Sánchez-Barredo et al. 2011). DIN was scarce relative to DIP, with DIN:DIP ratios consistently at or below the Redfield ratio of 16 (which is thought to approximate the threshold of P limitation for algae; Redfield et al. 1963) supporting the idea that temperate waters are typically N-limited systems (Hurd et al. 2014)

4.5.2 Carbon and nitrogen dynamics

Fluctuations in NO_3^- levels in seawater were largely reflected by the N content of seaweed thalli, with maximum % N (and C:N) corresponding with winter highs in ambient NO_3^-. Tissue % N of all species dropped dramatically between November and February when environmental levels of DIN were low, reflecting both depletion of internal N stores and dilution of tissue % N due to rapid growth facilitated by high summer irradiance (Abal et al. 1994). Although our sampling frequency did not allow us to accurately determine the lag between declines in ambient nitrate and depletion of N from algal tissues, most fucoids (Lehvo et al. 2005) and laminarians (Chapman & Craigie 1977) can store N for eight weeks or more. In contrast, *Macrocystis pyrifera* is thought to have a much more limited storage capacity and is therefore strongly influenced by ambient nitrate availability (Jackson 1982, Wheeler & Srivastava 1984, Shivji 1985, Zimmerman & Kremer 1986). This limited capacity for N storage was demonstrated by the large fluctuations in % N of *Macrocystis* tissue due to the absence of buffering N storage pools, while the threefold increases in % N between summer and winter demonstrates the highly efficient capacity of *Macrocystis* for N uptake under favourable conditions, which permits its characteristically rapid growth (up to 0.5 m day^{-1}; Neushul & Haxo 1963).

Seaweeds typically accumulate C over summer when irradiance and photosynthetic production is high, generating carbohydrate stores that permit rapid growth in the following spring (Stewart et al. 2009, Nielsen et al. 2014). While we didn't observe any clear seasonal patterns in % C of *Ecklonia* or *Phyllospora* at either location, the low carbon content of subsurface *Macrocystis* fronds in late autumn may indicate a reduction in irradiance below the critical value required for carbohydrate accumulation. Alternatively, given the largely perennial nature of *Macrocystis* in Fortescue Bay (Flukes pers. obs.), a reduction in tissue C content between summer and autumn may reflect the onset of C export by mature blades (Lobban 1978), while increasing tissue C over the winter months may indicate a decline in this export phase, potentially triggered by critically low irradiance.

Importantly, tissue C was consistent across depth ranges in *Ecklonia* indicating that the ability of this species to store carbohydrates was not influenced by light conditions. Moreover, carbohydrate storage did not affect tissue enrichment or depletion of $\delta^{13}C$. While growth and photosynthetic rate (i.e. productivity) were not directly measured, the consistency in % C of *Ecklonia* tissue across depths despite increases in the rate of carbon assimilation ($\delta^{13}C$) with decreasing depth suggests that shallow seaweeds were more productive than deeper thalli (see also Ramus et al. 1976). Alternatively (or additionally), shallow *Ecklonia* may translocate stored C away from older blades for new meristematic growth to compensate for higher levels of tissue loss due to greater wave exposure and grazing by herbivores (Nielsen et al. 2014).

The tissue ratio of C to N is frequently used as an indicator of N-limitation status in seaweeds (e.g. Hanisak 1983, Lapointe 1987, Frederiksen & Rueness 1989, Rico & Fernández 1996). Although this ratio is not always a robust measure of nitrogen concentration *in situ* because it is a function of both carbon and nitrogen metabolism, it can be interpreted in combination with % N to determine the nutritional status of seaweed (Hanisak 1979). A ratio of C:N greater than ~15 is thought to indicate N deficiency (Hanisak 1983), while an N content of ≥1.7% is required to maintain maximum growth in phaeophytes (Pedersen & Borum 1996). Values of C:N and % N determined by this study indicated that *Macrocystis* had sufficient N to sustain maximum growth throughout autumn and winter but was N-limited throughout spring and summer, while levels of N in *Phyllospora* (at both locations) remained below the critical threshold required for maximal growth year-round. The N-limitation status of *Ecklonia* varied slightly depending on whether C:N or % N was used as an indicator of

nutritional status, but Tasmanian seaweeds were only N-limited over summer whereas NSW thalli were permanently N-deficient. While seaweeds may persist indefinitely in an N-limited state, chronic N limitation typically reduces growth and productivity below the value it may otherwise reach when N is not limiting (Lapointe & Ryther 1979, Osborne & Geider 1986) due to a reduction in the synthesis of nitrogenous photosynthetic pigments (chlorophylls) and associated decline in photosynthetic yield (Turpin 1991).

Nitrogen limitation was less problematic for seaweeds in deeper water (30 m), where tissue N content was consistently > 2% and 1.5% for Tasmanian and NSW *Ecklonia*, respectively. Indeed, N-limitation typically only occurs under high light where the rate of carbon fixation relative to NO_3^- accumulation is relatively rapid (Hurd et al. 2014). A similar depth-related reduction in N limitation was observed by Nielsen et al. (2014) in *Saccharina latissima*, with the presence of pycnoclines during summer months suggested to be responsible for enhancing nutrient availability at depth. Minimal differences were detected in the nutrient composition of seawater between subsurface (2 m) and sub-mixed layer (15 m) samples, although in NSW during summer 2012 a water mass with vastly different nutrient (~3.2 µM NO_3^-) and temperature (16.8 °C) characteristics to surface water was detected as a distinct benthic layer below 15 m depth. Intrusions into shallower water of water masses typically found below 150 m depth are common along this area of coastline where the continental shelf is relatively narrow (Suthers et al. 2011). However, despite the presence of this seemingly anomalous water mass, the % N of *Ecklonia* at 30 m was not markedly different than that of thalli measured the previous summer, suggesting the intrusion was relatively recent and/or that the rate of N uptake by NSW thalli may saturate at relatively low nitrate concentrations (e.g. see Chapter 2 for *Phyllospora* N uptake). The greater N content of deep thalli at both locations is therefore likely a result of slower light-limited growth resulting in increased concentrations of tissue N for a given rate of uptake (Rosenberg & Ramus 1982).

4.5.3 Isotope signatures

The fraction of the heavy C isotope $\delta^{13}C$ present in seaweed tissues can be used to trace the species of inorganic C used for photosynthesis, with thalli restricted to CO_2 use typically having $\delta^{13}C$ values below -29‰, while those that also utilise HCO_3^- via active carbon concentrating mechanisms (CCMs) typically have values > -25‰ (Raven et al. 2002b). All three species monitored in this study appear to utilise a combination of diffusive CO_2 uptake

and active HCO_3^- uptake via a CCM, as indicated by $\delta^{13}C$ values maintained at approximately -15 to -20‰, -14 to -17‰, and -16 to -18‰ for *Ecklonia*, *Phyllospora* and *Macrocystis*, respectively. These values are within the range reported elsewhere for these species (Hepburn et al. 2011, Fox 2013). While *Phyllospora* and *Macrocystis* appeared to maintain similar levels of CCM activity across seasons, *Ecklonia* utilised more diffusive uptake of CO_2 as a C source over winter (particularly in Tasmania) when temperature and irradiance were reduced. This is likely due to the relatively lower light environment in which this species was measured (i.e. at depths of 10 and 30 m *vs.* 6 m for *Phyllospora* and 1 m for *Macrocystis*), where winter irradiance may have been insufficient to permit photosynthetic compensation for the more energetically expensive process of HCO_3^- uptake (Kübler & Raven 1994, Hepburn et al. 2011). Unsurprisingly, isotope data indicated that CCM activity was greater in shallow *Ecklonia* at each location (as per Fischer & Wiencke 1992), with Tasmanian seaweeds consistently maintaining a greater use of HCO_3^- (higher $\delta^{13}C$ values) relative to thalli at the same depth in NSW, despite being exposed to lower temperature (4-6 °C cooler) and irradiance for much of the year. However, the reverse was true for deep *Ecklonia* (i.e. $\delta^{13}C$ values for NSW *Ecklonia* > Tasmanian thalli), suggesting that additional factor(s) such as water motion or nutrient availability (potentially interacting with irradiance; Cornielsen et al. 2007), may influence C assimilation rates, or that other CCMs that do not alter isotopic values are used in combination with direct HCO_3^- uptake (Fernández et al. 2014). Another possibility is that varying degrees of lipid accumulation as an energy storage mechanism may also result in differentially depleted $\delta^{13}C$ values between seaweed groups (Sackett et al. 1974), but lipids were not measured by this study.

Signatures of $\delta^{15}N$ are typically used to trace food web dynamics (Fourqurean et al. 1997, Fredriksen 2003, Lepoint et al. 2004) or identify the importance of anthropogenic inputs of combined nitrogen sources to coastal habitats (Cohen & Fong 2005, Cornielsen et al. 2007, Lin & Fong 2008). Microalgae are known to preferentially uptake $\delta^{14}N$ over the heavy isotope $\delta^{15}N$, however this kind of fractionation appears not to occur in seaweeds (Cohen & Fong 2005). Much less is known about the ecophysiological implications of varying N isotope abundance compared to C isotopes, and while it may be related to the use of NH_3 *versus* NO_3^-, this has yet to be robustly demonstrated for macroalgae (Marconi et al. 2011). Therefore, the seasonal variability in tissue fractions of $\delta^{15}N$ observed across all Tasmanian seaweeds likely reflects variation in the isotopic signatures of local water masses (Sigman et

al. 2000), pointing to much more labile coastal oceanographic patterns in southeastern Tasmania than occurs at the site in NSW (which is exposed to EAC influence year-round).

4.5.4 Patterns in pigment content and photophysiology

All seaweeds in Tasmania demonstrated a strong pattern of increasing pigment concentration from a minima in February (summer) through to maximum levels in August-September (winter), coinciding with low temperature and irradiance. This is a common compensatory response to low ambient light levels, and typically occurs via increases in the number of reaction centres per unit biomass or thallus area (Ramus 1981, Fairhead & Cheshire 2004a). While Tasmanian seaweeds had high concentrations of all three measured pigments over winter, NSW seaweeds did not compensate for reduced irradiance in the same manner, with only chlorophyll *c* increasing in concentration over this time and no appreciable change in chlorophyll *a* or fucoxanthin. This suggests either that NSW seaweeds are unable to respond to lowered irradiance by increasing pigment synthesis in the same manner as Tasmanian thalli, or that it is energetically unnecessary to do (as the range in irradiance between summer maxima and winter minima at low latitudes is only half as great as that at higher latitudes). Conversely for deep and shallow thalli, while *Ecklonia* in NSW manifested the anticipated patterns of maintaining greater concentrations of pigments at depth (to compensate for a chronically low light environment; as per Durako et al. 2003, Fairhead & Cheshire 2004b), in Tasmania deep (30 m) *Ecklonia* had significantly lower pigment concentrations than shallower (10 m) individuals during winter when light levels were at their lowest.

Nutrient (particularly N) limitation has been shown to impair the synthesis of chlorophyll *a* in some laminarians (Wheeler et al. 1984) and fucoids (Stengel & Dring 1998), however ambient N concentrations were relatively high over winter in NSW and at depth in Tasmania, and a recent laboratory experiment with *Phyllospora* found no link between nitrate availability (within normal environmental ranges) and pigment synthesis (Chapter 2). The reduction in irradiance over winter in NSW may not be sufficient to justify the additional energetic cost of increasing the synthesis of light-harvesting pigments, and it appears that the very low irradiance reaching *Ecklonia* at 30 m in Tasmania may be insufficient to meet the energetic demands associated with the synthesis of large quantities of pigments (Hurd et al. 2014). The latter indicates that *Ecklonia* at depth in Tasmania are strongly C-limited and likely have an inherently restricted photosynthetic capacity. Regardless, it appears that both

Ecklonia and *Phyllospora* at the northern and southern margins of their range have different physiological strategies in response to seasonal fluctuations in irradiance.

Given the very low concentration of chl *c* relative to either chl *a* or fucoxanthin (more than an order of magnitude less in all species), this pigment likely plays a relatively minor role in light harvesting, and increases in its concentration during winter may be related to changes in the spectral quality, rather than quantity, of light (Barrett & Anderson 1977). In contrast, fucoxanthin – which has previously been assigned both a photoprotective (Ramus et al. 1977, Stengel & Dring 1998) and light harvesting (Gévaert et al. 2002, Fairhead & Cheshire 2004b) role in phaeophytes – was present at concentrations ~4 times greater than chlorophyll *a* and *c* combined, and increased by an order of magnitude from summer to winter in Tasmanian seaweed populations. The 3-4 fold increase in the ratio of fucoxanthin : chl *a* measured in thalli over winter demonstrates an enhanced photon absorptional capacity per unit area of thalli (Rosenberg & Ramus 1982, Fairhead & Cheshire 2004b), and indicates that fucoxanthin likely plays an important light harvesting role in low light-adapted *Ecklonia*, *Macrocystis* and *Phyllospora*,

Photosynthetic characteristics (as measured by PAM fluorometry) also showed strong seasonal patterns, as has been extensively documented in other seaweed systems (e.g. Smith et al. 1983, Flores-Moya et al. 1995, Lüder et al. 2002, Fairhead & Cheshire 2004b, Kim et al. 2004, Robeldo & Freile-Pelegrin 2005, Saroussi & Beer 2007a). Many fucoids (Niemeck & Mathieson 1978) and laminarians (Davison & Davidson 1987, Sakanishi et al. 1989, Fairhead & Cheshire 2004b) can compensate for seasonal changes in temperature and irradiance by adjusting their light harvesting efficiency and specific activity of Calvin cycle enzymes, which typically results in changes in the efficiency of PSII (F_v/F_m) and sub-saturating photon irradiance (E_k) (Cheshire et al. 1996, Fairhead & Cheshire 2004b). As this study was primarily interested in photophysiological differences of seaweeds between seasons, depths and locations rather than quantifying actual photosynthetic rates, we did not attempt to approximate actual ETR by using an absorption factor to represent the fraction of incident light absorbed by thalli (as recommended by Saroussi & Beer 2007b). However, the strong seasonality in pigment concentrations of Tasmanian seaweeds was clearly an important strategy for moderating extremes in irradiance and reducing the effects of "energy crises" in which antennae cannot capture sufficient photons for photosynthetic production to exceed respiratory demands (Falkowski & LaRoche 1991). Certainly for *Phyllospora*,

Tasmanian thalli demonstrated less seasonal changes in $rETR_{max}$ and E_k than NSW individuals despite that these seaweeds experience approximately double the annual range in irradiance. This capacity to photosynthetically compensate for environmental extremes is particularly important at high latitudes where low winter temperature and irradiance creates an energetic trade-off between light harvesting efficiency and effectiveness of inorganic carbon acquisition (Kübler & Raven 1995), and likely allows Tasmanian seaweeds to optimise their photosynthetic production across seasonal cycles.

The rate of electron transport (ETR) through PSII typically increases with temperature due to the temperature-specific activity of Calvin cycle enzymes (Raven & Geider 2003, Hurd et al. 2014). Therefore, in the absence of thermal compensation, electron transport rates will scale positively with increasing temperature up until the point that a critical temperature maximum is exceeded and photosystem functionality becomes impaired (Davison & Davidson 1987, Andersen et al. 2013, Chapter 2). While maximum photosynthetic capacity ($rETR_{max}$) and subsaturating light irradiance (E_k) coincided with high summer temperatures for *Phyllospora*, NSW *Ecklonia* (both depths), and deep Tasmanian *Ecklonia*; shallow Tasmanian *Ecklonia* and *Macrocystis* did not demonstrate summer peaks in $rETR_{max}$ and E_k as would be expected if photosynthetic activity simply scaled positively with temperature. Although nutrient limitation can reduce $rETR_{max}$ by interfering with pigment synthesis or PSII functionality (Flores-Moya et al. 1995, Falkowski & Raven 1997), distLM analysis did not identify nutrient availability as an important driver of multivariate photophysiology and thus it appears an unlikely cause of reduced photosynthetic capacity (at least for *Ecklonia*). Moreover, the reduction in F_v/F_m of all Tasmanian seaweeds over summer (often interpreted as an indicator of photosynthetic 'stress'; e.g. Karsten et al. 2001, Baumann et al. 2009, Oláh et al. 2010) demonstrates a reduction in photosynthetic efficiency, likely resulting from high light stress (Ralph & Burchett 1995, Cordi et al. 1997, Gévaert et al. 2002).

Under conditions of high irradiance, not all the light energy absorbed by pigments can be effectively utilised in photosynthesis. Seaweeds capable of employing photoprotective mechanisms may undergo ‘dynamic photoinhibition’ whereby excess light energy is dissipated through the xanthophyll cycle (Krause 1988, Demmig-Adams & Adams 1996, Hanelt et al. 1997), while those without photoprotective capacities may experience photo-oxidative damage to PSII reaction centres and other cellular components (Krause 1988). Seaweeds in this study were sampled at around midday (which represented peak or close to

peak irradiance) to reduce potential diurnal variability in photosynthesis (Belshe et al. 2008, Edwards & Kim 2010), with instantaneous PAR values over summer in Tasmania as high as 800 µmol photons m^{-2} s^{-1} (10 m depth, no canopy shading). Even assuming a ~90% reduction in light levels by the presence of a canopy (Fitzpatrick & Kirkman 1995, Wernberg et al. 2005), *in situ* PAR values were far greater than the subsaturating irradiance of Tasmanian seaweeds at any time of year (< 50 µmol photons m^{-2} s^{-1} for *Ecklonia*) and thus some degree of photoinhibition in summer is likely. While dynamic photoinhibition (photoprotection) typically results in increased E_k (as was observed for *Phyllospora*), shallow Tasmanian *Ecklonia* and *Macrocystis* demonstrated a reduction in E_k associated with low values of $rETR_{max}$ and F_v/F_m, which may instead indicate photodamage to PSII (Franklin et al. 1996). The morphology of *Phyllospora* is better suited to high irradiance than *Ecklonia*, with thick blades (less photosynthetic surface area) and extensive branching causing substantial self-shading which reduces exposure to potentially damaging levels of irradiance. That neither *Ecklonia* nor *Phyllospora* in NSW showed any signs of photoinhibition may indicate the presence of additional UV-absorbing 'sunscreen' pigments (Durako et al. 2003), or the maximum summer irradiance at the NSW site may simply not be sufficiently high (total daily PAR dose ~20% less than at the Tasmanian site) to induce photoinhibition in these seaweeds.

4.5.5 Decoupling of RNA:DNA ratios from other physiological processes

Growth (linear extension, biomass changes etc.) is frequently used as an indicator of 'health' or physiological success of seaweeds, as it is the end product of multiple simultaneously operating biochemical processes (Hasanuzzaman et al. 2013). However growth measurements of seaweed can be confounded by multiple levels of variability in blade erosion rates (Miller et al. 2011), grazer pressure (Toth et al. 2007) and the nature of growth (biomass accumulation *vs.* blade elongation; Nielsen et al. 2007), and does not account for diversion of energy resources towards other non-growth related processes. Ratios of RNA to DNA can be used as an alternative measure of 'growth potential', as rapidly growing organisms require more protein synthesis machinery (in the form of RNA-rich ribosomes) per unit biomass while DNA is a static index of cell number or biomass (Holm-Hansen 1969, Dortch et al. 1983). RNA:DNA ratios have been shown to closely mirror somatic growth in a wide range of taxonomic groups from bacteria (Kemp et al. 1993) to fish larvae (Catalán et al. 2006), zooplankton (Yebra et al. 2011), microalgae (Dortch et al. 1983) and higher plants (Reef et al. 2010a). While RNA:DNA ratios can be influenced by tissue concentrations of

both DNA and RNA, [DNA] is typically regulated by cell or genome size (e.g. Bennett 1976, Bennett & Smith 1976, Kapraun 2005, Knight et al. 2005) and is thought to be relatively environmentally invariant. However, we observed considerable variability in [DNA] of seaweeds across seasons, depths, and locations, supporting recent work (Chapters 2 & 3) that suggests DNA content of seaweeds is not static as is often assumed. The observed latitudinal and depth patterns in [DNA] were generally consistent with slower growth at low temperature and/or irradiance resulting in increased cell packing density and higher concentrations of nuclear DNA (Dortch et al. 1983). DNA concentrations in *Macrocystis* were an exception to this pattern, with peaks occurring in winter/spring (typically the period of maximum growth for this species; Wheeler & North 1981, Zimmerman & Kremer 1986, Brown et al. 1997) indicating that variability in [DNA] in *Macrocystis* may instead be associated with changes in plasmid (chloroplast, mitochondrial) DNA, e.g. increased density of chloroplasts in response to low winter irradiance.

If RNA concentration in seaweeds is positively associated with growth, high [RNA] should correspond with low [DNA] (and result in high RNA:DNA ratios). While shallow *Ecklonia* in NSW exhibited this pattern, the reverse was true for *Macrocystis* in Tasmania, with low [DNA] corresponding with low [RNA] and vice versa. Although RNA:DNA ratios were high in *Macrocystis* over summer months, this was driven predominantly by low [DNA] rather than high [RNA], similar to the findings from experimental studies with other similar species (Chapters 2 & 3). This suggests that high RNA concentrations in seaweeds may be associated with non-growth-related processes such as cellular repair (Kirst & Wiencke 1995), "futile cycling" associated with protective metabolic processes (e.g. Britto et al. 2001), or synthesis of compounds such as heat shock proteins (Vayda & Yuan 1994) or antioxidant defences (Davison & Davidson 1987, Sweetlove et al. 2002, Hasanuzzaman et al. 2013). Moreover, RNA:DNA ratios in *Ecklonia*, *Phyllospora* and *Macrocystis* appear to be unrelated to either photosynthetic activity or carbon metabolism (and therefore presumably growth), with the correlation between RNA:DNA ratios and photophysiological, nutrient and stable isotope characteristics <0.38 for all measured parameters (data not presented here). This decoupling of RNA:DNA ratios from nutrient uptake and photophysiology supports the observations of Reef et al. (2012) and Chapters 2 and 3 (this thesis) which currently provide the only other studies (to our knowledge) of RNA:DNA ratios in seaweeds.

4.5.6 Multiple environmental drivers of seaweed ecophysiology and latitudinal variation in susceptibility to climate change

Changes in the measured physiological characteristics of all three seaweed species were associated with seasonal variability in environmental conditions. Site (latitudinal) and depth-related patterns in ecophysiology contained in the combined multivariate suite of physiological characteristics were not always evident when considering parameters on their own. For example, while spatial and temporal variation in the univariate physiological characteristics of seaweeds did not always follow obvious patterns, distLM analysis unambiguously identified temperature (and irradiance, for *Ecklonia*) to be the most critical environmental predictor of differences in the multivariate physiology of seaweeds between seasons, latitudes and depths. Although best fitted distLM model for *Ecklonia* also included phosphorus and DIN:DIP, N was the limiting nutrient for growth so P availability was unlikely to have affected seaweed physiology directly. Instead, P concentrations (and DIN:DIP ratios) may be correlated with other unmeasured environmental variables such as turbidity (density of microalgal blooms or quantity of suspended sediment) which can indirectly affect *in situ* irradiance (e.g. Kregting & Gibbs 2006). Importantly, a substantial percentage (49%, 62% and 36% for *Ecklonia*, *Phyllospora* and *Macrocystis*, respectively) of variation was not accounted for by any of the environmental variables we measured, indicating unmeasured factors were affecting seasonal, latitudinal and depth patterns in algal ecophysiology. Important unmeasured factors may include spectral quality (Corzo & Niell 1991, Bischof et al. 2007), wave exposure (Fowler-Walker et al. 2006, Miller et al. 2011), epiphytic fouling (Hurd et al. 1994), grazer pressure (Dethier et al. 2005, Russell & Connell 2005), competitive interactions with other species (Ansell et al. 1998), or genetic variance between individuals (Kusumo & Druehl 2000).

Temperature is frequently identified as a primary driver of physiological processes in seaweeds (see review by Hurd et al. 2014), and increased water temperatures resulting from current and predicted levels of climate change will undoubtedly influence the growth (Mabin et al. 2013, Gaitán-Espitia et al. 2014), survival (Smale & Wernberg 2013, Mohring et al. 2014) and distribution (Breeman 1990, Wernberg et al. 2010, Smale & Wernberg 2013) of seaweeds. Physiological studies with *Ecklonia radiata* (Novaczek 1984, Mohring et al.) and *E. cava* (Serisawa et al. 2001) spanning different temperature regimes have demonstrated that the thermal tolerance and optima of seaweeds typically reflects the local climatic conditions

of the source population. A widely-held assumption is that marginal populations of seaweeds (at low latitudes) are likely to be more susceptible to temperature increases than their higher latitude counterparts, as they exist near the warm extent of the species' thermal tolerance (e.g. Harley et al. 2006, Wernberg et al. 2013). Indeed, a recent study by Mohring et al. (2014) demonstrated that cooler (high latitude) populations of *E. radiata* within its Australian distribution could withstand greater increases in water temperature than those growing closer to their upper thermal limits. However, thermal tolerance and/or optima studies do not account for the potentially interactive (additive, synergistic or antagonistic) effects of temperature increases in combination with other environmental stressors (e.g. Lotze & Worm 2002, Harley et al. 2006, Russell et al. 2009, Gaitán-Espitia et al. 2014).

While the results of our distLM analyses identified temperature as a primary driver separating patterns in multivariate ecophysiology in all three species (especially *Phyllospora*), PAR was also identified as an important environmental driver in *Ecklonia*. Moreover, we found strong evidence of stress (measured as F_v/F_m) over summer (during peak irradiance) in shallow *Ecklonia* at high latitude, while lower latitude populations and those in deeper water (exposed to a similar temperature regime) were seemingly unaffected. The occurrence of stress during summer at high latitude was less pronounced for *Phyllospora*, possibly because this species occurs only in the shallows and is physically better adapted to withstand high irradiance due to thick blade tissue and extensively branched morphology that creates a strong self-shading effect. Any thinning of *Phyllospora* canopy at high latitudes is therefore likely to have particularly severe negative impacts on conspecifics due to positive feedback between canopy thinning (reduced shading) and survival of remaining thalli. Hence, while temperature increases may, in isolation, impact more severely on low latitude seaweed populations near the upper margins of their thermal tolerance (Mohring et al. 2014; but see also Chapter 2), the combination of climate change-driven oligotrophy and increases in water temperature with existing seasonally stressful levels of irradiance may have disproportionately severe effects on *Ecklonia* and (to a lesser extent) *Phyllospora* at higher latitudes.

4.5.7 Conclusions

These experiments demonstrate that the physiology of *Ecklonia*, *Phyllospora* and *Macrocystis* is strongly influenced by seasonal variation in environmental conditions, but the nature of these patterns varies subtly with depth and latitude. Seaweeds at lower latitudes

appear better adapted to deal with simultaneous increases in temperature and irradiance over summer, but whether this indicates adaptive differences across the latitudinal range of *Ecklonia* and *Phyllospora* or simply reflects the lower magnitude of seasonal changes (particularly in irradiance) at low latitudes is not clear. Low nutrient availability over summer due to the presence of oligotrophic EAC waters appears to affect seaweeds equally across their latitudinal and depth ranges, while temperature and irradiance emerge as far more dominant regulators of seaweed physiology (particularly for *Ecklonia* and *Phyllospora*). The lack of support for the GRH across latitudes likely reflects the considerable ability of *Ecklonia* and *Phyllospora* to store nutrients and their capacity for efficient N uptake even at very low environmental nitrate concentrations (Chapter 2), while increased carbon limitation with increasing depth restricts the photosynthetic activity (and presumably growth) of seaweeds such that the potential for nutrient limitation is lessened with increasing depth. Moreover, deep seaweeds at high latitudes showed no evidence of the summer light stress that characterised shallower thalli, suggesting that the slowed growth and reduced nutrient demand at depth may ameliorate the negative impacts of predicted environmental change, and that this environment may provide a refugia from future stressful conditions in a manner similar to that which has been observed for other seaweed species (Graham et al. 2007a). Importantly, the absence of a positive cline in nutrient limitation with increasing latitude or depth, in combination with no clear relationship between ambient nutrient availability, RNA synthesis, or any measured physiological process, suggests the GRH does not apply to seaweeds in this system. In the context of predicted climate change, we suggest that *Ecklonia* and *Phyllospora* will be affected similarly by increased temperature and reduced nutrients across their latitudinal range (or potentially impacted more severely at low latitudes; Mohring et al. 2014) but that these stressors will interact synergistically with high summer irradiance at high latitudes so that southern seaweeds (particularly those in shallow waters) will be more adversely affected by climate change than is widely assumed.

Chapter 5 Thinning of kelp canopy modifies understory assemblages: the importance of canopy density

5.1 Abstract

Kelp forests in southeastern Australia form canopies that support complex understory assemblages. Predicted levels of climate change in this region are likely to impact on the health and distribution of these forests, potentially resulting in large-scale reductions in canopy cover. This study determined the impacts of a permanent reduction in the canopy cover of the dominant kelp in this region, *Ecklonia radiata*, on the structure of understory algal and sessile invertebrate community assemblages. Changes in assemblages were determined over 12 months in three treatments: unmanipulated, 33% canopy reduction and 66% canopy reduction. Clearance treatments were maintained to simulate the predicted effects of long-term climate-driven canopy reduction. Thinning of *E. radiata* canopy (especially 66% loss) caused a shift towards a foliose algal-dominated understory, with an associated loss of sponges, bryozoans, and encrusting algae. Canopy loss homogenised existing patchiness in understory assemblages, and high recruitment of *E. radiata* occurred at both levels of thinning. A 66% reduction in kelp canopy increased understory community diversity, but did not affect species richness. Thus, changes to understory assemblages occurred in a density-dependent manner, with 66% canopy loss required to alter the structure of assemblages at the community scale. Changes at this scale were subtle but important (with stability attributed to a combination of biogeography and resistance to perturbation driven by high diversity); and indicate that partial loss of kelp canopy under future climate change scenarios will shift understory communities towards a foliose algal-dominated state, which has important implications for sessile invertebrates and potentially future recruitment of kelp.

5.2 Introduction

Macroalgal beds are a dominant feature of temperate waters worldwide, and provide the ecological foundations of most temperate marine reef ecosystems (Steneck & Johnson 2013). The vertical structure of subtidal kelp forests creates a complex three-dimensional habitat that modifies abiotic factors such as light (Gerard 1984, Reed & Foster 1984, Irving et al. 2004), hydrodynamics (Kennelly 1989, Duggins et al. 1990, Connell 2003) and sedimentation (Eckman et al. 1989, Wernberg et al. 2005), permitting the development of complex understory assemblages (Dayton et al. 1992, Clark et al. 2004). These communities are often characterised by high levels of biodiversity and endemism (Dayton 1985) and create an important food source and microhabitat for other reef-associated organisms (e.g. Andrew et al. 1998, Edgar et al. 2004).

An increasing threat to kelp and other canopy-forming macroalgae is increasing ocean temperatures driven by climate change processes (e.g. Dayton & Tegner 1984, Wernberg et al. 2010, Wernberg et al. 2011a). Ocean warming, particularly when coupled with other (potentially synergistic) stressors such as nutrient depletion and/or eutrophication (Russell & Connell 2007), ocean acidification (Wernberg et al. 2011b), increased frequency of storm or El Niño events (Dayton & Tegner 1984) and strong grazing pressure (Vanderklift et al. 2009), is expected to erode the resilience of kelp beds via direct physiological effects (Wernberg et al. 2011a) and reduction of successful recruitment (Mabin et al. 2013, Mohring et al. 2013a). While kelp, as with most organisms, can undergo physiological and structural changes (acclimatize) to at least partially adapt to chronically warm environments (Staehr & Wernberg 2009), this comes at the ecological cost of reduced resilience which, in the context of sustained climate-driven warming, will ultimately lead to range contractions and decreased abundance of kelp as it approaches the limits of its physiological tolerance (Wernberg et al. 2010, Poloczanska et al. 2013).

Temperate reefs in southeastern Australia are dominated by the large brown kelp *Ecklonia radiata*. With a larger depth (4-50+ m) and latitudinal (27.5-43.5 °S) range than any other canopy-former in Australia (Steinberg & Kendrick 1999), *E. radiata* is temperate Australia's most important habitat-forming seaweed. Southeastern Australia is also one of the most rapidly warming areas in the world at ~3.8 times the global average rate, and is predicted to establish as the most intense 'hotspot' of ocean warming in the southern hemisphere

(Ridgway 2007). This increase in water temperature is driven by increased wind stress in the Southern Ocean, resulting in longer and stronger incursions of the warm East Australian Current (EAC) into waters off eastern Tasmania as a result of eddy propagation (Cai 2006, Ridgway 2007). Water temperatures in the southwest Tasman Sea have warmed by more than 2.2 °C over the past century (Ridgway 2007) so that summer maxima now routinely reaches temperatures of ~18 °C in southeastern Tasmania, which is nearing the upper thermal tolerance limits (~22 °C) for successful reproduction of *E. radiata* in Tasmania (Mabin et al. 2013). Predicted levels of warming in this region over the next century (> 3 °C; Lough 2012) are likely to impact on the density and distribution of *E. radiata* via reduced growth (Hatcher et al. 1987), survival (Wernberg et al. 2013), success of microscopic stages (Mabin et al. 2013), and reproductive output (Mohring et al. 2013a), which may ultimately result in widespread decline in density and thinning of adult canopies with concomitant impacts on associated understory assemblages.

The role of kelp in regulating understory communities has been demonstrated by canopy removal experiments (Johnson & Mann 1988, Valentine & Johnson 2005, Toohey et al. 2007); and by tracking community responses to natural disturbances that create gaps in canopies through processes such as storms (Thomsen et al. 2004), herbivore grazing (Johnson & Mann 1993, Ling 2008), or localised warming events (Valentine & Johnson 2004, Smale & Wernberg 2013, Wernberg et al. 2013). In general, algal and invertebrate communities beneath kelp canopies are more diverse than those on reefs lacking a canopy (Dayton 1985, Watt & Scrosati 2013), which are typically dominated by few species of coralline algae (Melville & Connell 2001). Nonetheless, despite their importance in promoting biodiversity, canopy-forming seaweeds can also have a strong limiting effect on understory algae by reducing access to light and space (Goodsell & Connell 2005, Wernberg et al. 2005) and via mechanical abrasion of the benthos (Kennelly 1989, Connell 2003), all of which inhibit recruitment and post-recruitment survivorship (Toohey et al. 2007, Wernberg & Connell 2008). A partial reduction of canopy cover may therefore relax the competitive effects of the canopy-former (Dayton 1985, Kennelly 1987b, Edwards 1998) allowing the recruitment and proliferation of understory species (Kennelly 1989, Toohey et al. 2007).

Macroalgal clearance studies examining understory community responses have typically focused on the effects of total canopy removal (Emmerson & Collings 1998, Edgar et al. 2004), particularly in the context of intense herbivory (Johnson & Mann 1993, Edgar et al.

2004, Ling 2008) or natural disturbances that effectively lead to total clearance of canopy-forming species (Valentine & Johnson 2004, Wernberg et al. 2013). In a climate change context, total canopy loss may only be realistic in situations such as El Niño (Dayton & Tegner 1984) or extreme warming events. For example, a recent warming event in Western Australia reduced *Ecklonia radiata* cover by almost 50% (Wernberg et al. 2013) and completely eliminated the fucoid *Scytothalia dorycarpa*, irreversibly reducing the northern extent of the latter species by ~100 km (Smale & Wernberg 2013). However, in areas that are not at the warm extreme of physiological tolerance for kelp, populations are more likely to be able to withstand periodic extreme warming by making physiological, structural, and metabolic adjustments (Wernberg et al. 2010); thus a more realistic scenario is a gradual reduction in canopy extent. While a small number of partial clearance/disturbance experiments have been conducted (see Kennelly 1987b, Wernberg & Connell 2008, Araújo et al. 2012), these have focused on the recruitment of the disturbed species or on recovery of understory assemblages through time after a single manipulation of canopy. Importantly, we could not locate any previous studies that have applied and maintained a partial canopy clearance over a prolonged period of time. Understanding how understory algal and sessile invertebrate communities associated with kelp may respond to permanent canopy thinning is critical for predicting the impacts of future climate change-driven threats on temperate reef communities.

This work assesses the idea that climate change in southeastern Australia will impact on the structure of understory community assemblages by reducing the cover of *Ecklonia radiata* canopy, but that the nature of this impact may vary depending on the extent of canopy loss. To simulate permanent climate change-driven reductions in kelp density, we thinned *E. radiata* canopy by 33% and 66% for 12 months and determined: (1) changes in understory algal and invertebrate community structure; and (2) effects of canopy thinning on *E. radiata* recruitment. Although the ecological responses of understory communities to canopy removal may vary depending on both the identity of the canopy-forming species and the geographic context of the ecosystem (reviewed by Santelices & Ojeda 1984), this study provides an important first step in quantifying the effects of partial canopy loss in the context of climate-driven pressures on temperate reef communities. We show that sustained canopy loss of *E. radiata* in southeastern Australia affects understory communities in a density-dependent manner, and that the sessile invertebrate component of understory assemblages on Tasmanian rocky reefs may be replaced by a foliose algal-dominated state under future climate change.

5.3 Materials and Methods

5.3.1 Study site and experimental set-up

The effect of *Ecklonia radiata* canopy on the structure of understory communities was assessed in a canopy thinning experiment undertaken on a shallow rocky reef in Fortescue Bay, Tasman Peninsula (43.123°S, 147.976°E) between 18th March 2011 and 16th March 2012. Fortescue Bay is characterised by moderate-relief boulder reef with extensive beds of *E. radiata* from a depth of ~6m down to 15+ m where the reef-sand interface occurs. The study reef was chosen specifically for the homogeneity of its boulder substratum, moderate wave exposure, and uniformity of depth. Nine plots (5 m by 5 m) were haphazardly placed within stands of well-developed *E. radiata* canopy at a depth of ~9-11 m. Treatment plots were separated from each other by ~5-10 m and positioned at least 1 m from any gaps in *E. radiata* canopy.

Three treatments were applied to experimental plots: control (0% canopy removal), low (1/3rd canopy removal, hereafter 33%), or high (2/3rd canopy removal, hereafter 66%), with $n = 3$ plots for each treatment. This equated to densities of 8.9, 6.2 and 3.1 plants m^{-2} for control, 33%, and 66% canopy thinning treatments, respectively. No plots were totally cleared of *Ecklonia radiata*, as this is an unlikely outcome under predicted climate change scenarios in Tasmania. Treatments were ordered randomly across the nine plots. Although the experimental plots were 5 x 5 m, response variables were only measured from the inner 3 x 3 m part of each plot which was demarcated by 8 mm UV-resistant polypropylene rope, providing a 1 m treated buffer zone to minimise edge effects. Previous work with *E. radiata* has demonstrated that 2 x 2 m areas were sufficiently large to prevent shading of the centre of plots by surrounding canopies (Kennelly 1987b). Clearance treatments were administered by divers removing whole adult *E. radiata* plants (including stipes and holdfasts) by hand to reduce the density to 33 or 66% of the plot's original density. Plants were removed in a regular manner, i.e. 'every third plant' for 33% canopy thinning, or 'two in every three plants' for 66% thinning treatments. Plants were classified as adult only if they were deemed to contribute significantly to canopy shading, with a threshold of holdfast to blade tip length ≥ 50 cm used to distinguish (arbitrarily) between juvenile and adult plants. The density of mature *E. radiata* plants within each plot was assessed using five randomly placed 1 m^2 quadrats.

5.3.2 Experimental maintenance and assessment of community structure

Surveys of algal community structure were conducted immediately prior to canopy thinning, and at 6 and 12 months after administration of initial thinning treatment. Understory communities were assessed in five randomly placed 0.25 m^2 quadrats in each plot, and the cover of macroalgae, sessile invertebrates, and bare substratum quantified using 49 regularly spaced points of intersecting wire. Large foliose understory alga that lay prostrate across the substrata were moved aside to determine the cover of smaller turfing algae, hence it was possible to arrive at a total percentage cover in a given plot greater than 100%. Where a species could not be accurately identified to at least family level (i.e. an immature or damaged specimen), it was allocated only to Phyla or functional group. This made up < 10% of species and < 0.5% total cover on all occasions. Groups containing multiple morphologically indistinguishable species were classified into functional groups (e.g. encrusting red algae, filamentous red turfing algae, geniculate coralline algae; see Table 1). Adult *E. radiata* densities were quantified at ~2 month intervals and adult thalli removed as necessary to maintain the appropriate canopy treatments. Any individuals of the range expanding diadematid grazing sea urchin *Centrostephanus rodgersii* were removed, as their presence was unrelated to canopy cover and their destructive grazing could mask effects of canopy thinning treatments.

5.3.3 Statistical analysis

As we were not specifically interested in documenting seasonal changes in community structure, we focussed our analyses on similarities among treatments before (i.e. pre-manipulation) and after 12 months of maintained canopy thinning. For this reason, and because we were *a priori* interested to determine similarity across treatment plots before the manipulations were undertaken, 'time' was not used as a factor in analyses. Instead, we ran separate tests for the start of the experimental period (pre-manipulation) and at the end of the monitoring period (following 12 months of maintained canopy thinning). This approach avoids potential issues with seasonality in species abundances (Kennelly 1987b) because both sampling occasions are at the same time of year. While we could not negate the possibility of inter-annual variability in community structure, any such changes are likely to affect all treatments similarly.

Changes in understory community structure under different levels of canopy thinning were determined using multivariate analyses before and after 12 months of the treatment being established. While adult *Ecklonia radiata* did not comprise part of the understory community and were not included in the community analyses, juvenile *E. radiata* were recorded in point intersect counts. We conducted two separate analyses with and without juvenile *E. radiata* to ascertain whether changes in understory community structure were due to *E. radiata* recruitment. Canonical analysis of principal coordinates (CAP) was used to distinguish differences in multivariate community structure using Bray-Curtis similarity matrices after data were square root transformed (Clarke 1993). The 10 most influential taxa contributing to differences in assemblages across treatment groups, as identified by Spearman correlation coefficients, were examined graphically. Permutational multivariate analysis of variance (PERMANOVA) with pre-planned contrasts was used to test the null hypothesis that community assemblages did not differ among treatment groups at the commencement of the experiment, or after 12 months of canopy manipulation. A nested PERMANOVA design was used (with 999 permutations) at each of the two sampling occasions with the factors 'treatment', and 'plot' nested within 'treatment'. The software package PRIMER-E (v 6.1.12) was used for all multivariate analyses.

The effects of canopy thinning on recruitment of *E. radiata*, and on understory community richness (algal and invertebrate species combined), Shannon Wiener diversity (H', H_{max}, Evenness; Molles & Cahill 1999), understory algal cover, and total understory assemblage cover (i.e. non-bare areas of substratum, algal and invertebrate species combined); were examined using nested ANOVA, with the factors 'treatment' and 'plot' nested within 'treatment'. Two separate ANOVA were run for each dependent variable; one at the start, and one at the end of the experiment. All data were first checked for conformity to the assumptions of homoscedasticity and normality, and transformations to stabilise variances (if required) were determined by the relationship between group SDs and means (Draper & Smith 1998). Tukey's HSD *a posteriori* multiple range tests were performed where significant differences were detected among treatments.

5.4 Results

Understory assemblages were diverse in all canopy treatments, with a total of 52 algal species/taxa and four sessile invertebrate taxa identified (Table 5.1). The most abundant species at commencement of the experiment were *Sonderopelta coriacea* (15.3%), *Rhodymenia* spp. (10.6%), non-geniculate corallines (10.2%), encrusting red algae (7.4%), and geniculate corallines (5.2%). Bryozoans (9.4%) and sponges (7.9%) were also a prominent feature of assemblages. However, at the end of the experiment, invertebrates (sponges, bryozoans) and encrusting algal complexes had declined in the high clearance treatment while certain terete and foliose species (*Griffithsia elegans, Hemineura frondosa*) increased in cover. This change was not observed in the low clearance or control treatments (Fig. 5.1). *Halopteris* spp. and *Plocamium leptophyllum* also increased in plots subject to high level canopy clearance, but abundance of these species was highly spatially and temporally variable.

Table 5.1 List of macroalgae and sessile benthic invertebrate taxa identified in understory assemblages beneath *Ecklonia radiata* canopy in Fortescue Bay, Tasmania.

Chlorophyta
- *Caulerpa flexilis*
- *Caulerpa geminata*
- *Caulerpa trifaria*
- *Codium fragile*
- *Ulva* spp.

Phaeophyta
- *Acrocarpia paniculata*
- *Carpoglossum confluens*
- *Carpomitra costata*
- *Colpomenia* spp.
- *Cystophora* spp.
- *Dictyota* spp.
- *Ecklonia radiata*
- *Halopteris* spp.
- *Phyllospora comosa*
- *Sargassum* spp.
- *Seirococcus axillaris*
- *Xiphophora gladiata*
- *Zonaria / Lobophora* complex

Rhodophyta
- *Ballia callitricha*
- *Callophyllis rangiferina*
- *Camontagnea oxyclada*
- *Carpopeltis phyllophora*
- *Ceramium excellens*
- *Craspedocarpus ramentaceus*
- *Delisea plumosa*
- Encrusting red algae
- Filamentous turfing red algae
- *Gelidium asperum*
- Geniculate coralline algae
- *Gigartina pinnata*

Rhodophyta (continued)
- *Glaphrymenia pustulosa*
- *Griffithsia elegans*
- *Halicnide similans*
- *Hemineura frondosa*
- *Hymenena* spp.
- *Hypnea racementacea*
- *Kuetzingia canaliculata*
- *Laurencia elata*
- *Lenormandia marginata*
- Non-geniculate coralline algae
- *Peyssonnelia novaehollandiae*
- *Phacelocarpus* spp.
- *Plocamium angustum*
- *Plocamium leptophyllum*
- *Polyopes constrictus*
- *Rhodophyllus multipartita*
- *Rhodophyllis* spp. (other)
- *Rhodymenia* sp. 1
- *Rhodymenia* sp. 2
- *Sarcothalia crassifolia*
- *Sonderopelta coriacea*
- *Thamnoclonium dichotomum*

Porifera
- Encrusting sponge

Cnidaria
- Hydroid spp.

Bryozoa
- Bryozoan spp.

Chordata
- Ascidean

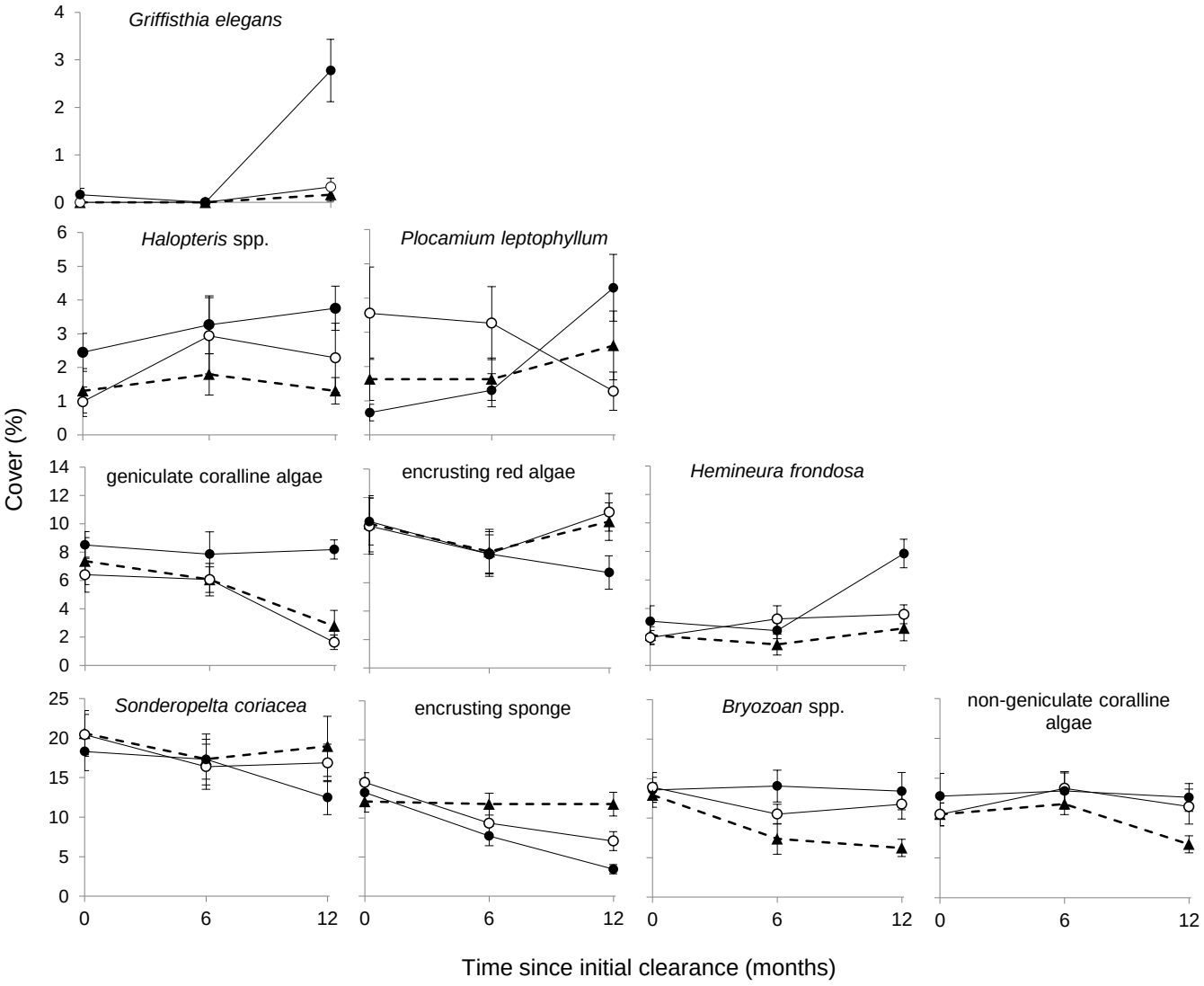


Figure 5.1 Effects of *Ecklonia radiata* canopy clearance on the percentage cover of selected algal and invertebrate taxa. The taxa displayed here were identified by Spearman correlation coefficients as the top 10 most influential contributors to differences in assemblages across treatment groups. Treatments were (▲) control; (○) low (33%) clearance; and (●) high (66%) clearance (n = 15 quadrats per treatment, pooled across plots). Data are means ± SE.

5.4.1 Multivariate community structure

Community assemblages did not differ between treatment groups at the commencement of the experiment (Table 5.2; Fig. 5.2). After twelve months of maintained canopy thinning, significant differences in the structure of understory communities were detected among treatment groups (Table 5.2; Fig. 5.2). Initial CAP plots from all time periods represented on a single set of axes (data not presented here) also showed an overall shift in the structure of

communities before clearance, and 12 months after canopy manipulations. However, this affected all treatments equally (i.e. characterised reef-scale inter-annual variability), hence t = 0 and t = 12 months data are presented on separate plots for clarity (Fig. 5.2). The separation of assemblages between manipulated (both low and high) and control plots was driven by changes in abundance of a number of different taxa; ranging from coralline and encrusting algal species, to sessile invertebrates and erect foliose algae (Fig. 5.1). Pairwise tests could not identify which treatments differed (control *vs*. high p = 0.089; control *vs*. low p = 0.999; low *vs*. high p = 0.090), but the effect of treatment was clearly driven predominantly by a separation between high clearance plots and low clearance/control plots (Fig. 5.2B). When juvenile *E. radiata* were also considered as a component of the understory community, low canopy thinning plots became distinguishably different from controls but were still separated from high thinning treatments in CAP space (Fig. 5.2D); although as with the previous analysis pairwise tests could not determine significance due to plot within treatment effects (p = 0.043, Table 5.2B).

Table 5.2 Nested PERMANOVA comparing among-canopy thinning treatments for understory community structure both excluding and including juvenile *Ecklonia radiata* (based on Bray Curtis similarity of % cover using a square-root transformation). Factors are 'treatment' (3 levels of *E. radiata* canopy thinning: control; low, 33%; high, 66%) and 'plot' nested within 'treatment'. Significance is indicated in **bold**.

Source	*df*	start (t = 0 months)			end (t = 12 months)		
		MS	*F*	*p*	MS	*F*	*p*
Excluding							
Treatment	2	774	0.571	0.918	2725	2.20	**0.029**
Plot (Treat)	6	1355	1.54	**0.008**	1238	1.34	**0.034**
residuals	36	881			925		
Including							
Treatment	2	778	0.569	0.928	3099	2.55	**0.016**
Plot (Treat)	6	1367	1.55	**0.003**	1215	1.33	**0.043**
residuals	26	881			913		

The significant plot within treatment effect (Table 5.2) indicated spatial variability in understory community structure occurred on a scale of 10^0-10^1 m. Examination of individual subsamples in CAP plots at the commencement of the experiment (Fig. 5.2A, C) revealed significant "clumping" of subsamples within plots, demonstrating the small spatial scale of patchiness in community structure. After 12 months of maintained canopy thinning (low and high), subsamples became more similar across replicate plots within each treatment group, and this homogeneity increased with increasing levels of clearance (Fig. 5.2B, D).

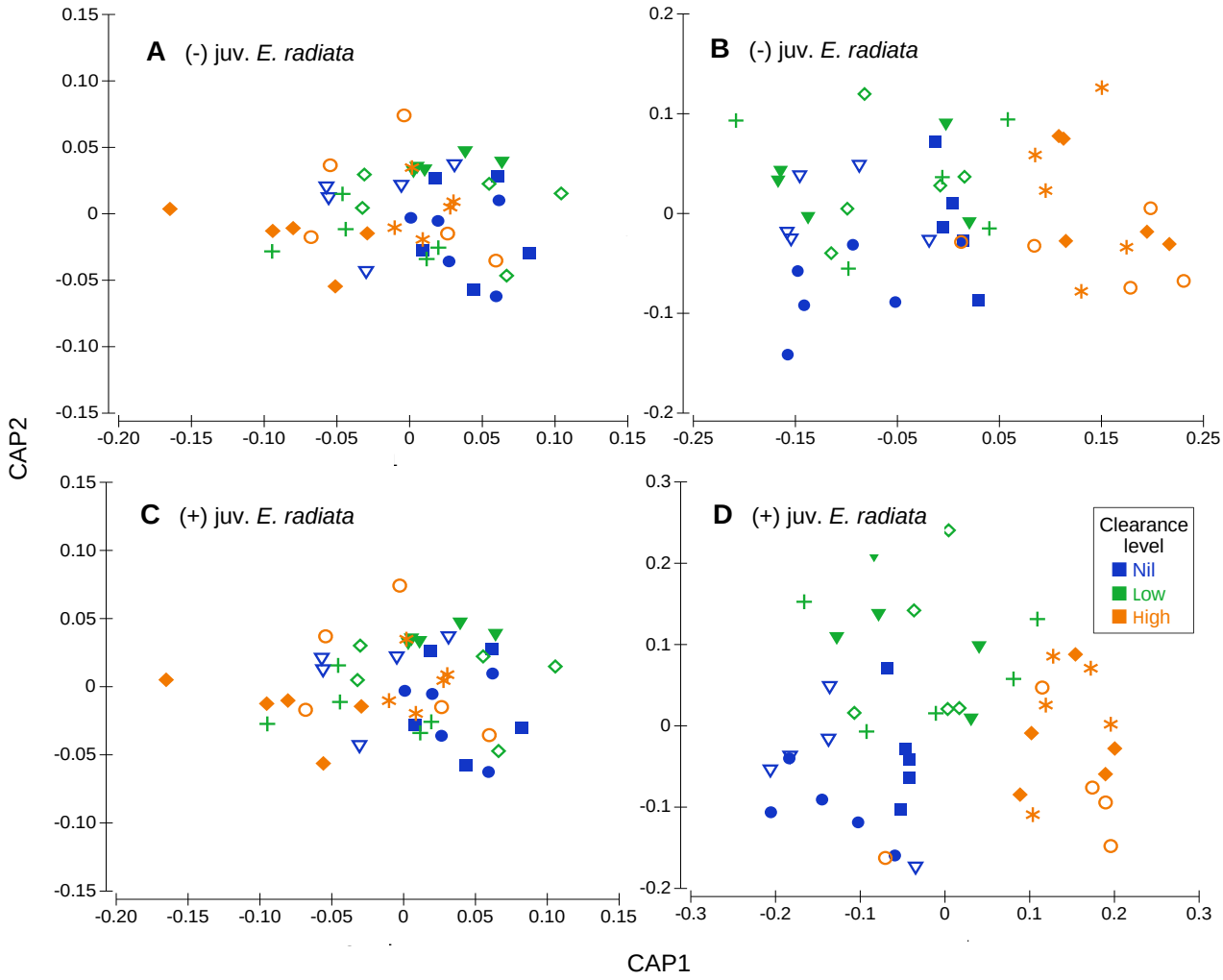


Figure 5.2 Structure of understory algal and sessile invertebrate community assemblages **(A, C)** before; and **(B, D)** after 12 mo of maintained *Ecklonia radiata* canopy thinning. Canonical analysis of principal coordinates of assemblages (% cover data) **(A, B)** excluding (–); and **(C, D)** including (+) juvenile *E. radiata*. Symbols identify individual plots (n = 3), with each point representing a quadrat subsampled within that plot (5 quadrats plot^{-1}). Canopy manipulation treatments were nil: no clearance; low: 33% clearance; high: 66% clearance (n = 15).

5.4.2 Univariate community parameters

There was no initial difference in total assemblage cover, algal cover, species richness, or Shannon-Wiener diversity indices among treatment groups before the canopy clearances were applied ($p > 0.08$ for all parameters; analyses not shown, Fig. 5.3). Twelve months of sustained canopy thinning had no effect on species richness or maximum diversity (H_{max}); however, it did result in a significant increase in overall algal cover, Shannon-Wiener diversity (H'), and evenness (Table 5.3). In a similar pattern to that observed in multivariate space, high clearance treatments had significantly higher levels of both H' ($p = 0.007$) and evenness ($p = 0.015$) than either low or no clearance treatments (Table 5.3; Fig. 5.3C, D). The percentage cover of understory algae was elevated in both high and low clearance treatments relative to control plots ($p = 0.009$), experiencing an 11% and 15% increase to 93% and 101% cover, respectively (Table 5.3; Fig 5.3G). This was due to a shift from sessile invertebrate to algal cover (Fig. 5.3G, see also Fig. 5.1), as absolute cover of combined invertebrate and algal assemblages remained high and unchanged across all treatment groups for the duration of the experiment (~93-98% at all times, Fig. 5.3F). In general, plots of these parameters over time show a clear divergence of communities in high clearance treatments from control and low clearance (for H' and evenness) treatments after 12 months of maintained thinning (Fig. 5.3C, D).

Table 5.3 Nested ANOVAs comparing among-canopy thinning treatments (control, low, high) for 7 parameters relating to understory algal and invertebrate community structure: the density of juvenile *Ecklonia radiata*, understory community richness and diversity (3 indices: Shannon-Weiner *H*', H_{max}, evenness); algal cover (3-dimensional); and total cover (non-bare areas occupied by invertebrate and algal assemblages). * $0.01 < p < 0.05$; ** $p < 0.01$.

Source of variation	*df*	Density juv. *E. radiata*		Species richness		Diversity (*H*')		H_{max}		Evenness		% algal cover (3-D)		% total cover (2-D)	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Treatment	2	172	**6.85****	15.0	2.88	0.264	**5.78****	0.089	3.23	0.0086	**4.71***	0.0581	**5.39****	0.00029	0.477
Plot (Treat)	6	31.5	1.25	7.67	1.47	0.099	2.17	0.044	1.60	0.0024	1.31	0.0118	0.387	0.00099	1.64
residuals	36			5.20		0.046		0.028		0.0018		0.0108		0.00060	

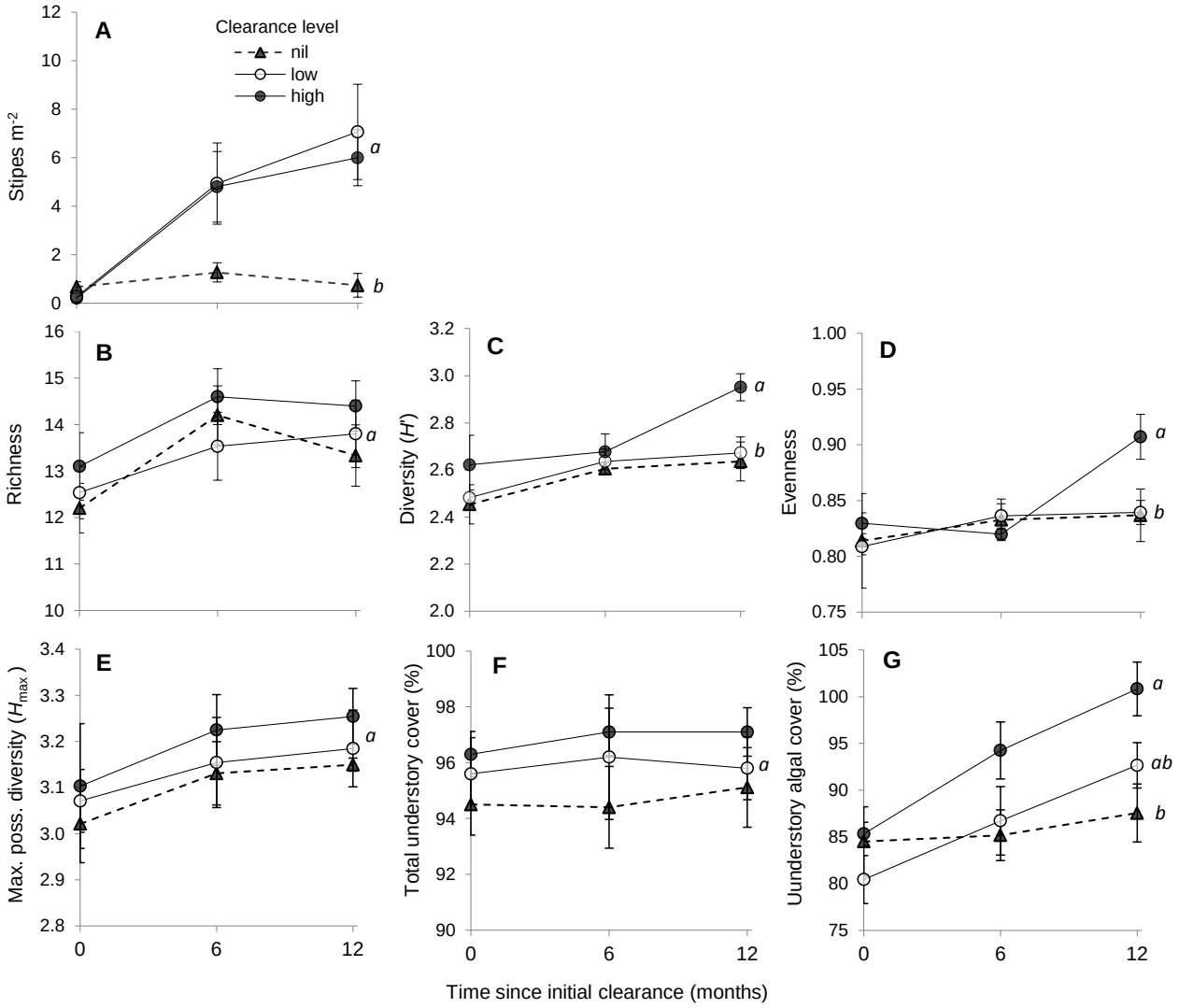


Figure 5.3 Mean changes in **(A)** juvenile *Ecklonia radiata* density; **(B)** understory species richness; **(C)** Shannon-Weiner diversity (*H*'); **(D)** species evenness; **(E)** maximum possible diversity (H_{max}); **(F)** total cover of substratum by understory species (non-bare areas); and **(G)** cover of understory algae, over 12 mo of maintained *E. radiata* canopy thinning. Treatments were nil: no clearance; low: 33% clearance, and high: 66% clearance (n = 15). Data are means ± SE. Letters to right of plots indicate Tukey's HSD groupings of treatments for each response variable ($p \geq 0.05$).

5.4.3 Kelp recruitment

Both low and high clearance treatments resulted in a significant increase in recruitment of juvenile *Ecklonia radiata* (Table 5.3; Fig. 5.3A). A strong recruitment pulse occurred within the first six months of canopy thinning in both low and high clearance plots. This elevated level of recruitment continued for the following six months despite an increasing proportion of these 'early' recruits being classified as adults at the time of 12 month surveys (Fig. 5.3A). The increased frequency of clearance of 'adult' *E. radiata* necessary to maintain canopy density at appropriate levels from six to 12 months is further evidence for elevated recruitment (Fig. 5.4). Unlike whole community structure, there was no small-scale patchiness observed in *E. radiata* recruitment between plots ($p > 0.3$, Table 5.3), indicating that recruitment either occurred relatively homogenously on this spatial scale, or that any patchiness in recruitment was at too small (< 3 m) or too large (>10s of m) a scale to be detected by our sampling.

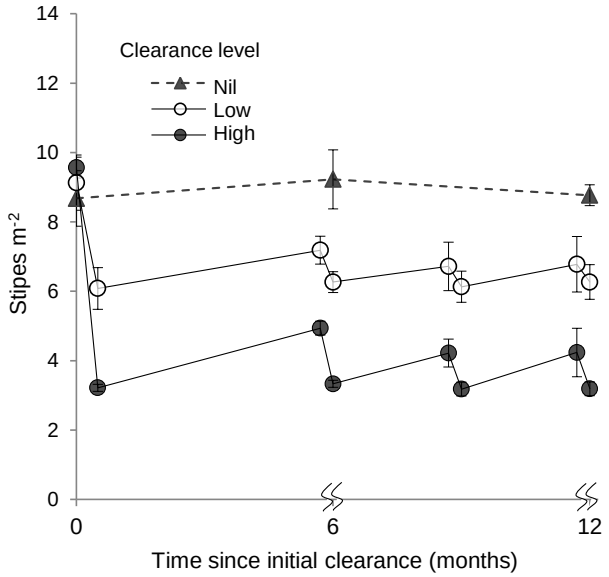


Figure 5.4 Mean changes in the density of adult *Ecklonia radiata* over 12 mo of *E. radiata* canopy thinning. Treatments were nil: no clearance; low: 33% clearance, and high: 66% clearance (n = 15). Data are means ± SE.. Decreases in low and high clearance treatments at ~6, 9 and 12 mo indicate where canopy has been thinned by divers to maintain clearance treatments. Axis breaks are used to show kelp density before and after thinning treatment was applied on each occasion.

5.5 Discussion

5.5.1 Density-dependent effects of canopy reduction, and nonlinearities in community responses

A reduction in the cover of *Ecklonia radiata* canopy resulted in significant changes in the structure of understory community assemblages, and elevated recruitment of kelp. The shift in overall community structure between cleared and control treatments was driven predominantly by an increase in filamentous and foliose algae such as *Hemineura frondosa*, *Halopteris* spp. and *Griffisthia elegans*; and a concomitant loss of sponges, bryozoans and encrusting and calcareous algae. Although high levels of patchiness occurred in understory communities on the scale of $10^0 – 10^1$ m, analysis of individual species responses suggested that community structure typically varied little between control and low canopy thinning treatments, with high (66%) levels of canopy thinning required to cause a significant change in the structure of understory assemblages.

Perhaps the most dramatic effect of macroalgal canopy on the understory environment occurs via shading, with estimates of light absorbance by kelp canopies ranging from ~75% (Edwards 1998) to > 95% (Reed & Foster 1984, Kennelly 1989, Wernberg et al. 2005). Wernberg et al. (2005) demonstrated that kelp canopies reduce irradiance in a nonlinear manner, with irradiance decreasing up to a density of 2 sporophytes 0.25 m^{-2}, beyond which denser canopies caused no further reduction in light at the understory level. A similar nonlinear effect of *E. radiata* canopy density on understory irradiance occurs at our site, with photosynthetically active radiation (PAR) levels beneath the canopy subject to high levels of thinning (mean daily irradiance ± SE of 2.17 ± 0.16 and 0.50 ± 0.03 mol photons m^{-2} day^{-1} for January and June, respectively); consistently greater than those beneath low clearance (1.04 ± 0.12 and 0.32 ± 0.19 mol photons m^{-2} day^{-1}) and unmanipulated (0.84 ± 0.12 and 0.34 ± 0.02 mol photons m^{-2} day^{-1}) canopy, which were indistinguishable and ~70% less than in areas completely cleared of kelp (7.44 ± 0.43 and 0.75 ± 0.04 mol photons m^{-2} day^{-1}; M. Tatsumi *unpub. data*). A consequence of nonlinearities in the physical environment with varying levels of canopy cover may be nonlinearities in the response of understory communities. The fact that high levels of canopy thinning were required to effect significant change in the structure of understory assemblages suggests that, under low levels of canopy thinning, the density of the remaining canopy is sufficient to maintain a similar light (Gerard

1984, Reed & Foster 1984, Irving et al. 2004, Wernberg et al. 2005), hydrodynamic (Kennelly 1989, Duggins et al. 1990, Connell 2003) and sediment (Eckman et al. 1989, Wernberg et al. 2005) environment to that beneath intact canopies.

It has yet to be determined (due to a paucity of studies on the effects of partial canopy removal) whether the reduction in kelp canopy required to effect a community-scale change depends on the proportion of existing canopy removed (i.e. relative change), or whether an absolute threshold of canopy cover exists below which understory assemblages will be affected. Regardless, the effects of kelp canopy removal is likely to depend on the initial density of adult kelp on a reef, which can be highly spatially variable (Schiel & Foster 1986, Kendrick et al. 1999) depending on local environmental factors (e.g. Wernberg & Thomsen 2005, Fowler-Walker et al. 2006). Indeed, encrusting coralline algae appear to be affected by kelp clearance in a nonlinear manner depending on the initial kelp density, with responses to experimental clearance occurring in different directions depending on the initial cover of kelp (Melville & Connell 2001). Moreover, the initial composition of understory assemblages likely also plays an important role in community-scale resistance to canopy perturbation. For example, Kennelly (1987b) found some understory species responded to a 25% thinning of canopy, while others were unaffected by canopy reductions less than 50%. Clearly, further research on the density-dependent effects of canopy thinning is required; however, in the context of predicted climate change-driven reductions in *Ecklonia radiata* canopy, it appears that the response of understory assemblages will depend strongly on the extent to which canopy is reduced, and thus on how well *E. radiata* can acclimate and adapt to a changing environment (Wernberg et al. 2010).

5.5.2 Effects of canopy loss on the diversity and structure of understory communities

Natural disturbance to kelp forests or canopy clearance experiments have previously demonstrated large understory community-scale transformations (e.g. Kennelly & Underwood 1993, Melville & Connell 2001, Toohey et al. 2007, Wernberg et al. 2013). A frequently reported effect of canopy clearance is a dramatic increase in species richness that is thought to be due in increases in light and space resources (e.g. Kennelly 1989, Toohey et al. 2004, Wernberg et al. 2005, Toohey et al. 2007). While we observed an increase in diversity (Shannon-Wiener H' index) after 12 months of high level canopy thinning, this was

driven primarily by a reduction in abundance of dominant taxa rather than increased richness. Specifically, a reduction in cover of sessile invertebrates and encrusting algae (which were abundant at the start of the experiment) increased the evenness of the community without affecting species richness. We cannot isolate whether this assemblage shift was driven by a decrease in sessile invertebrates and encrusting algae because the loss of canopy created an unfavourable environment for these taxa, or by the competitive dominance of foliose algal species upon release of light and space resources. Wernberg et al. (2013) suggest a combination of both these effects, proposing that a reduction in kelp canopy facilitates proliferation of understory algae but also causes a decline in encrusting invertebrates that are associated with kelp (see also Wernberg & Connell 2008).

The marked decrease in the abundance of sessile invertebrates and encrusting red algae with increasing levels of *Ecklonia radiata* canopy thinning are consistent with the observations of Kennelly (1989) and Wernberg and Connell (2008). In the present study, the abundant encrusting algae *Sonderopelta coriacea* decreased with increasing canopy loss similar to the declines in *Peyssonelia* spp. observed by Kennelly (1989). These taxa are structurally and functionally very similar and species in this family often co-occur in shaded environments on the reef matrix due to their obligate low light requirements (Häder et al. 1998). Similarly, many sponges host photosynthetic symbionts which are sensitive to elevated light levels (Lemloh et al. 2009). Kelp canopies are known to reduce sedimentation on understory communities by trapping sediment "rain" and scouring sediment from the substrata (Kennelly 1989, Wernberg et al. 2005). Organisms that we observed to decline following canopy clearance (particularly the filter-feeding bryozoans and ascidians) may be susceptible to a synergy of increased sediment loading and light, as the mature recruitment stages of many invertebrate larvae is photonegative (Thorson 1964) and their developmental success is reduced by sedimentation (Irving & Connell 2002). While Connell (2003) demonstrated that the negative effects of macroalgal canopy abrasion can outweigh the positive effects of reduced light and sedimentation for sessile invertebrates, we found invertebrate assemblages to be a dominant (> 25% cover) component of communities beneath unmanipulated canopy, and their observed decline (to < 10% cover) under high levels of canopy thinning suggests a climate-driven loss of canopy will likely reduce the abundance and biodiversity of these taxa.

5.5.3 Relative stability of understory communities

A previous study across multiple sites in this region reported no significant change in understory community structure following *Ecklonia radiata* canopy clearance (Edgar et al. 2004). The authors proposed that the lack of community-scale responses may be due to the high diversity and density of understory species; both of which were similar to values observed in our experiment. Given the magnitude of community-scale changes following canopy reduction reported elsewhere (often a halving of species richness or a complete shift from foliose- to encrusting coralline algae-dominated communities; Melville & Connell 2001), the shifts we observed here were relatively modest. As proposed by Edgar et al. (2004), the apparent stability of assemblages in southeastern Tasmania may be driven (at least in part) by high diversity which is known to enhance community resistance to perturbation and recovery from disturbance (e.g. Johnson et al. 1996, Tilman 1996, Aquilino & Stachowicz 2011). Indeed, the high species richness of 12-16 species per 0.25 m^2 quadrat in the present study is similar to that reported by Sánchez and Fernández (2005) on intertidal assemblages in Spain and by Toohey et al. (2007) in Western Australia, and is typical of the speciose nature of reef systems around southern Australia (Bolton 1994, Kerswell 2006). Although we observed only subtle community-level changes following canopy reduction, they were important nonetheless and contrast with the absence of community effects observed by Edgar et al. (2004). This may be due to the depth of our study site (~10 m) where sponges and bryozoans were an abundant (13.3% and 13.7% of initial plot cover, respectively) component of assemblages and strongly influential in separating the structure of communities beneath thinned *vs*. unmanipulated canopy, whereas sessile invertebrates are less abundant at 3-6 m and were not recorded by Edgar et al. (2004). Furthermore, only administering a single clearance treatment (100% canopy loss) once at the commencement of the experiment may not allow relaxation of *Ecklonia radiata* competitive-dominance, thereby reducing the opportunity for understory species to utilise available light resources (Dayton 1985, Kennelly 1987b).

5.5.4 Importance of biogeography, scale, and localised patchiness in determining the effects of canopy thinning

Recent work by Wernberg et al. (2013) reports on changes in community assemblages following an opportunistic thinning of *Ecklonia radiata* canopy resulting from an extreme

warming event in Western Australia (WA). Loss of kelp canopy in this context appeared to represent an irreversible change, and Wernberg and his colleagues (2013) reported a dramatic shift to an ecologically depauperate state of simple algal turf mats. Connell and Russell (2010) also observed a similar response in South Australia (SA) with a proliferation of turf-forming algae in the absence of *E. radiata* canopy, although they linked the final composition of algal assemblages to an interaction between nutrient levels and the presence of grazers. In these systems, the establishment of turfs facilitated by canopy loss effectively excludes all other species by inhibiting the further recruitment of canopy-formers (Kennelly 1987b, Russell & Connell 2005). However, dense turf mats do not appear to develop following canopy removal in eastern Tasmania (Edgar et al. 2004, Valentine & Johnson 2005) in the same manner as occurs in WA and SA, highlighting the importance of biogeographic context in determining community responses to canopy loss. The shifts in community structure we observed are likely to be conservative in that they represent a response to manipulation of a single species in the community matrix, whereas multiple understory species are likely to be directly affected by climate change via warming water temperatures in addition to the indirect effects of canopy loss (which may act synergistically). In this sense, the observations of Wernberg et al. (2013) are particularly important because they provide observations on multiple species affected by a warming event. However, it is also important to note that many of these species were on the threshold of their physiological tolerance and hence caution must be exercised in extrapolating the observed effects to higher latitudes away from range margins.

The scale of canopy clearance is an important factor determining the nature of detectable community responses (Kennelly & Underwood 1993, Kendrick 1994, Emmerson & Collings 1998). For example, larger clearances involve a greater proportion of the total pool of available species and are more likely to detect changes in communities at the reef scale, but most studies focus on relatively small (several square metres) areas of clearance (e.g. Kennelly & Underwood 1993, Emmerson & Collings 1998, Sánchez & Fernández 2005), as this a common size of naturally-occurring clearances caused by storms or grazers. In the context of long-term climate change, canopy thinning would be expected to occur over 10s to 100s of km, but ongoing maintenance of experimental treatments at this scale is clearly unfeasible. Importantly, we were able to detect patchiness within communities at the scale of $10^0 – 10^1$ m, with quadrats within plots sharing more similar community structure than those in adjacent plots. A similar effect was reported by Kendrick et al. (2004) and Toohey et al.

(2007) and may be due to variability in the scale of disturbances (e.g. selective removal of individual plants by grazers *vs*. extensive canopy denudation in storm events), timing of disturbances in relation to phenology, and spatial extent of spore dispersal (Dayton 1985). Although the reef we selected for this study was relatively homogenous, differences in the size of boulders and degree of shading would cause microhabitat heterogeneity (e.g. in local hydrodynamics and the light environment) among plots. However, this patchiness appears not to alter the nature of the community response to canopy thinning, and the thinning itself actually homogenises this spatial variability. Thus, canopy thinning appears to reduce small-scale patchiness in community structure at the 'plot' scale, effectively removing the effects of past disturbance history.

The ecological responses of communities to canopy removal will not necessarily be geographically consistent, as demonstrated by the varying responses of understorey assemblages to total canopy removal experiments in southern Chile and the North Pacific (Santelices & Ojeda 1984). Moreover, the effects of canopy clearance are likely to depend on the identity of the dominant canopy-forming species in a region, as this influences the initial composition of understory assemblages (e.g. see differences in assemblage composition between Reed & Foster 1984, Santelices & Ojeda 1984, Kennelly 1987b, Kendrick et al. 1999, Clark et al. 2004, Araújo et al. 2012). The treatments applied in this study were not spatially replicated across multiple sites, as the degree of within-site replication and the fine scale at which we assessed understorey assemblages made replication across multiple sites and/or regions unfeasible. Edgar et al. (2004) identified this issue of scaling and applied a broad level of spatial replication (over 50 km) to their *E. radiata* canopy manipulation study, and found that variation in macroalgal assemblages between sites was much greater than that associated with experimental treatments, and that the nature of canopy clearance effects had a minimal, but spatially consistent effect. Moreover, Kennelly and Underwood (1993) demonstrated geographic consistencies in the effects of *E. radiata* canopy disturbance on understory community structure in New South Wales across a range of different spatial scales. Thus, while we recognise that a lack of site replication does restrict the general applicability of results obtained here and that caution must be exercised in extrapolating results to other regions dominated by *E. radiata* (or indeed other canopy forming species), the patterns we observed are unlikely to be unique to southeastern Australia. Clearly, spatial replication of sustained 'pressure'-type (partial) canopy clearance experiments is required

across a broad geographic scale, and we recommend the development of standardised methodology to assist with comparing the outcomes of such studies.

5.5.5 Effects of canopy removal on kelp recruitment

The high recruitment by *Ecklonia radiata* following a reduction in canopy cover supports the findings of previous studies on *E. radiata* and other kelp (e.g. Johnson & Mann 1988, Kennelly & Underwood 1993, Kendrick 1994, Emmerson & Collings 1998). In general, canopy removal is associated with enhanced survival, settlement and growth of recruits, but the timing of clearances (both seasonal and in relation to species phenology) has been highlighted as important in influencing survival of new recruits (Kennelly 1987a, Kennelly & Underwood 1993). We observed elevated levels of recruitment throughout both summer and winter and although plots contained a dense foliose-dominated understory, these assemblages did not appear to inhibit growth of *E. radiata* sporophytes in the same manner as turfing algae (Emmerson & Collings 1998, Russell & Connell 2005).

The enhanced kelp recruitment we observed may have been due to increased settlement success, or recruits may have been present prior to canopy manipulations (as dormant/seed bank stages) and simply experienced rapid growth upon opening of canopy gaps. Regardless, it suggests that the competitive ability of juvenile *Ecklonia radiata* when light resources are released may be particularly strong in southeastern Tasmania, potentially as a compensatory mechanism to facilitate growth in a low light climate. While Valentine and Johnson (2005) observed very little recovery of *E. radiata* in eastern Tasmania following removal of a competitively dominant invasive kelp, their site was characterised by high sediment loading which is known to inhibit recruitment success (Devinny & Volse 1978, Eriksson & Johansson 2003). Here, we observed a proliferation of juvenile *E. radiata* at both levels of clearance, indicating a small reduction in canopy cover is sufficient to allow large increases in zoospore settlement and/or gametophytic growth, or possibly a release of microscopic stages from developmental dormancy that can occur when conditions are unfavourable for growth (e.g. Hoffmann & Santelices 1991, Carney & Edwards 2006, Carney & Edwards 2010). This finding does not necessarily imply equal or greater recruitment under scenarios of total canopy clearance, as environmental conditions in the absence of canopy (high light and sedimentation, reduced hydrodynamic dampening) can be unfavourable for seaweed

recruitment (Wernberg & Connell 2008). Moreover, canopy clearance may indirectly affect kelp recruitment by altering the abundance of invertebrate grazers (positively or negatively; see Konar & Estes 2003, Christie et al. 2009), which can have complex effects on kelp microscopic stages and recruitment success (e.g. Henríquez et al. 2011). Finally, total canopy loss may result in proliferation of turf-forming algae in place of diverse foliose-dominated communities (although this may depend on biogeography, as previously discussed), which can strongly negatively affect growth and/or settlement success of microscopic stages (Emmerson & Collings 1998, Russell & Connell 2005).

5.5.6 Summary

We have demonstrated that a reduction in cover of *Ecklonia radiata* canopy causes subtle but important changes to the structure of understory community assemblages. If future climate change causes significant thinning of the *E. radiata* canopy, understory communities may shift towards a more foliose algal-dominated state with fewer sponges, bryozoans, and encrusting/calcareous red algae. Moreover, we have shown that the understory communities associated with *E. radiata* vary in a density-dependent and nonlinear manner. However, the specific way in which a community will respond to canopy thinning is likely to depend on the initial composition of the community (including the individual species' physiological tolerances to elevated light and sediment), the biogeographic context of the system, and the extent of canopy thinning. Given the high species diversity and spatial coverage of assemblages documented in this study, we suggest that community assemblages beneath monospecific *E. radiata* stands in southeastern Tasmania are resistant to external perturbations. Future work should focus on spatial replication of sustained canopy thinning across the latitudinal range of *E. radiata*, and examining the effects of varying levels of canopy reduction to identify potential critical thresholds of canopy loss and nonlinearities in community responses.

Chapter 6 Synthesis: Assessing the impacts of environmental change on seaweeds: uncertain ecological interpretations and the danger of proxies

6.1 Abstract

Seaweeds play a central role in structuring rocky reef communities in temperate zones worldwide. Ongoing environmental change driven by climatic factors and direct anthropogenic activities poses an increasing threat to habitat-engineering seaweeds, and a central focus in ecological research is predicting the way in which current and future levels of environmental change will impact on their performance and distribution. Despite the growing importance of studies relating ecological performance with environmental change, the seaweed ecophysiological literature is increasingly reliant on the use of physiological indicators as proxies for growth and productivity. This review explores some of the limitations of using physiological proxies in place of traditional growth measurements, including the issues associated with ambiguity in interpretation, the dangers of generalising observations across algal taxa, and the assumptions that must be made to extrapolate physiological data to infer ecological conclusions. Even if the effects of complex environmental change on the ecological performance of seaweeds can be determined precisely, there are considerable additional uncertainties regarding both the adaptive capacity of seaweeds relative to current and predicted rates (and levels) of change, and how this will manifest ecologically at the community scale. We encourage researchers to continue using fundamental techniques for ecologically oriented studies until more robust connections can be developed between multiple physiological proxies and growth outcomes, and to reserve the use of additional physiological indicators as complementary to elucidate the mechanisms underpinning seaweed responses to environmental change.

6.2 Synthesis

Foundation, or 'ecosystem engineering' species play a critical role in regulating communities and ecological processes through the creation, modification, and/or maintenance of habitat. The role of ecosystem engineers in determining species richness at the landscape scale through modulation of resource availability has increasingly been recognised in terrestrial (e.g. Casas-Crivillé & Valera 2005), aquatic (e.g. Wright et al. 2002) and marine (e.g. Ling 2008, Kimbro et al. 2014) ecosystems worldwide. In the marine environment, seaweeds modify the abiotic environment beneath the canopy, changing hydrodynamic, light, scour, and sedimentation regimes (Gerard 1984, Connell 2003, Toohey et al. 2004, Wernberg et al. 2005). Seaweeds also provide structurally complex habitat that supports high levels of biodiversity and endemism (Dayton 1985, Steneck & Johnson 2013), thus any change in their density or distribution is likely to have large flow-on effects for associated organisms (Connell 2007, Ling 2008, Wernberg et al. 2013, Flukes et al. 2014). It follows that knowledge of the factors that influence growth, reproduction and survival of habitat-forming seaweeds, and the extent to which these species can respond to environmental change via acclimatisation, adaptation, or migration (Harley et al. 2012), is of fundamental importance in understanding the dynamics of temperate rocky reef communities.

Seaweeds are highly susceptible to physical and chemical changes in the marine environment, with survival, growth and reproduction significantly affected by abiotic factors such as water movement (Blanchette 1997, Wernberg & Thomsen 2005, Miller et al. 2011), temperature (Davison 1991, Clarke 2003, Mohring et al. 2014), nutrient supply (Figueroa et al. 2009, Hurd et al. 2014), pH (Diaz-Pulido et al. 2012, Gaitán-Espitia et al. 2014), desiccation (Kim & Garbary 2007, Chu et al. 2012) and irradiance (Altamirano et al. 2004, Toohey et al. 2004, Mata et al. 2006). Environmental characteristics (including biotic interactions) naturally vary in space and time, however direct anthropogenic activity and the effects of climate change are increasingly influencing the strength and/or direction of many environmental factors such that seaweeds are currently exposed to rates and levels of warming (Wernberg et al. 2011b), ocean acidification (Connell & Russell 2010), pollution, eutrophication (Connell 2007), alien species invasions (Ling 2008) and habitat destruction unprecedented on ecological time scales (see reviews by Feely et al. 2004, Harley et al. 2006, Rabalais et al. 2009). Not surprisingly, recent climate-driven range contractions have already been documented in many species of habitat-forming seaweeds in Australia (Johnson et al.

2011, Wernberg et al. 2011a), Europe (Müller et al. 2009, Philippart et al. 2011), South Africa (Bolton et al. 2012), and other temperate zones worldwide (see reviews by Breeman 1990, Wernberg et al. 2011a).

Linking ecological performance with environmental change

The need to understand the likely ecological costs of predicted environmental change on seaweeds, both at the individual and population level, is being increasingly recognised (Morton et al. 2009). A recent synthesis by Harley et al. (2012) provides a comprehensive review of the environmental factors that affect seaweed physiology, growth, reproduction and survival. However, our current understanding of the relationship between ecological performance and environmental drivers is largely based on a poorly synthesised combination of climatological and physiological studies.

Attempts to better link seaweed physiology with current (and predicted) climatic factors have used both targeted laboratory experiments and quantitative field observations. While laboratory studies are useful to elucidate the mechanisms of physiological response(s) to environmental change, relatively few examine the effects of multiple variables simultaneously (presumably due to the logistical difficulties associated with ballooning factorial designs) and thus often fail to account for potential interactions between multiple stressors (Lotze & Worm 2002). For example, algal turf cover was shown to decrease with increasing levels of CO_2 under ambient nutrient conditions while the reverse was true under elevated nutrients (Russell et al. 2009), and increased pCO_2 only reduces the survivorship of kelp spores when temperature is also elevated (Gaitán-Espitia et al. 2014). Moreover, laboratory studies can have limited ecological relevance as the culture conditions are generally optimal (except for the factor under study) which may not elucidate behaviour under suboptimal field conditions (Neushul 1981). Field studies are generally more ecologically relevant than laboratory experiments as they typically document the response of seaweeds to natural climate anomalies such as El Niño events (e.g. Dayton et al. 1999b), periodic upwelling (e.g. Anderson et al. 1996) or episodic warming (e.g. Wernberg et al. 2013), and extrapolate observed responses to predicted future conditions. However, changes in multiple environmental factors (temperature, nutrient levels, water movement) are usually confounded in large-scale climatic events (e.g. Bolton & Levitt 1987, Valentine & Johnson 2003) and thus complicate interpretations to derive a mechanistic link between changing environmental conditions and the physiology of affected species.

Determining the effect of environmental change on species' distributions frequently focuses around the use of bioclimate envelope models (BEMs) (e.g. Austin 2002, Guisan & Thuiller 2005, Araújo & Luoto 2007), which relate field observations of geographic distributions with environmental predictor variables to forecast future distributions into new or modified habitats. While BEMs have been used to forecast the broad-scale impacts of climate change on seaweeds within biogeographic regions (Wernberg et al. 2011a, Bartsch et al. 2012), these predictions rely on the assumption that that models developed in one portion of a species' range (e.g. low latitude 'rear edge' populations) can be extrapolated, either in space or time, to the new novel conditions (e.g. future high latitude 'leading edge' populations) which is not always ecologically plausible. Recent work suggests that the likelihood of successful predictions by BEMs can be increased with knowledge of the physiological characteristics of the study species, including factors such as thermal tolerance, energy balance, and respiratory dynamics (Woodin et al. 2013). Thus, while climate change will clearly result in poleward migration of many seaweeds (Wernberg et al. 2011a, Bartsch et al. 2012, Poloczanska et al. 2013), precise predictions of the timing and spatial extent of range shifts requires a robust physiological understanding of the physiological mechanisms limiting a species' distribution.

Use of physiological metrics as 'proxies' for ecological performance

Determining the response of seaweeds to a changing environment essentially involves two distinct levels of assessment. The first and most important in an ecological context is growth, survival, and reproductive success (hereafter all mention of 'ecological' performance refers to these three processes), as these factors ultimately determine the distribution and persistence of seaweeds within their environment. The second is understanding the mechanisms of how changing environmental conditions affect physiological processes in seaweed, ultimately culminating in the measured ecological response.

For seaweed research with an ecological focus, growth and/or productivity is ultimately what is important at the level of individual thalli (however it is measured; e.g. linear extension, change in biomass or thallus area, oxygen evolution or C-fixation; Falkowski & Raven 1997, Wernberg et al. 2010, Nielsen et al. 2014). However, direct measurements of absolute or net growth can be difficult to obtain, particularly in broad-scale field studies where continually locating and measuring tagged individuals across a large geographic range is logistically challenging. As a result, *in situ* growth studies are typically conducted on a relatively small

spatial scale (Altamirano et al. 2000, Fairhead & Cheshire 2004a, Miller et al. 2011), or short and/or infrequent temporal scale (Peckol et al. 1994, Aguilera et al. 1999). Similarly, for measurements of productivity, field studies determining O_2 evolution or CO_2 exchange require the use of incubators to minimise environmental variability (Littler & Arnold 1980) which rapidly becomes logistically challenging when dealing with large macroalgae. This has led to an emerging trend of using logistically simpler measurements of physiological metrics as so-called 'proxies' for growth and productivity.

The rapid rise in the use of physiological proxies has been driven at least in part by the falling cost and technological advances in sophisticated equipment and techniques. One piece of equipment responsible for a rapid gain in momentum of *in situ* seaweed research was the advent of a fully submersible pulse-amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Germany), which utilises the quenching of chlorophyll fluorescence to measure aspects of the photophysiology of Photosystem II (PSII) rapidly and non-destructively under natural conditions (critical for many subtidal field studies that use divers to sample seaweeds *in situ*). Similarly, the growing accessibility of spectrometers has resulted in increasing use of isotopic fractionation as an indicator of carbon metabolism (e.g. Hepburn et al. 2011, Raven & Beardall 2014), while rapid advances in molecular biology have recently led to the use of RNA and DNA concentrations and ratios in seaweeds to inform on aspects of physiological and biochemical processes (Reef et al. 2012; Chapters 2-4).

The development of sophisticated physiological measurement techniques has permitted rapid growth and advances in seaweed ecophysiological research. Chapters 2-4 of this thesis provide several case studies demonstrating the use of physiological proxies in laboratory and field studies of macroalgae, and offer examples of ecological interpretations that can be derived in combination with other physiological indicators (including growth; Chapters 2 & 3). However, caution must be exercised when interpreting physiological metrics exclusively to infer ecological performance, as this extrapolation typically requires the conception of a number of assumptions. This review provides a complementary overview of some of the potential limitations of using physiological proxies to infer ecological growth and performance. It is not intended to comprehensively review all available physiological proxies for macroalgal growth, but rather to explore several commonly used proxies and discuss their potential limitations. Case studies are used as examples throughout to illustrate the use and limitations of physiological proxies, and a final synthesis provides an overview of the current

state of seaweed ecophysiological research and highlights important areas for future investigation.

Issues with using proxies: variable interpretation of performance indicators

The first potential issue associated with the use of physiological indicators in place of direct growth measurements is that the ecological interpretation of proxies can vary considerably between different studies. In some cases, this demonstrates the versatility of a measurement in informing on multiple aspects of seaweed physiology. For example, the fractionation of carbon isotopes (i.e. the proportion of $^{13}C/^{12}C$, denoted as $\delta^{13}C$ relative to an international standard) in seaweed tissue can be used to trace the source of dissolved inorganic carbon (DIC) from the surrounding water column or growth media (Young et al. 2013). However, values of $\delta^{13}C$ can also represent the relative accumulation of carbohydrates *versus* lipids in algal tissue (as lipids are isotopically more depleted than carbohydrates; Post et al. 2007). A third valid and highly useful application of $\delta^{13}C$ is as an indicator of carbon concentrating mechanisms in algae (Raven & Beardall 2014), with higher (less negative) values of $\delta^{13}C$ indicating a greater use of the isotopically more enriched C-source HCO_3^- via a CCM (Raven & Beardall 2003).

Unfortunately, variation in the ecological interpretation of physiological indicators between different studies can just as frequently be incorrect, as physiological proxies are not unambiguous in the same manner as direct growth measurements. To continue with the example of carbon isotopic fractionation, another interpretation of $\delta^{13}C$ is as a reflection of, or predictor for, photosynthetic rate (e.g. Raven et al. 1995, Hu et al. 2012, Vanderklift & Bearham 2014). This idea assumes that the fractionation of C isotopes decreases as photosynthetic rate increases (leading to less negative $\delta^{13}C$ values), as an increased demand for C results in a reduced selectivity for ^{12}C allowing a greater proportion of the available C to be used. In reality, however, CO_2 in the thallus boundary layer is replenished at a constant rate, and the scenario of localised depletion within the diffusion boundary layer could only occur when the flow is low (<20 mm s^{-1}) and extremely homogenous (Cornwall et al. 2014), or when the metabolic activity of the seaweed increases the pH of the boundary layer microenvironment to ~9.0 or more (which would typically only occur within very dense surface canopy-forming kelp beds; Cornwall 2013). This interpretation of $\delta^{13}C$ has gained traction following several published studies documenting observations of positive correlations between $\delta^{13}C$ and irradiance/temperature, and between photosynthesis and

irradiance/temperature, leading to the inference that photosynthetic rate can be predicted by $\delta^{13}C$. While $\delta^{13}C$ values may be positively correlated with photosynthetic rates, the relationship is clearly not causative, and the exclusive use of $\delta^{13}C$ values in place of direct productivity measurements would likely result in significant over- or under-estimations of an alga's growth potential.

Issues with proxies: limited ability to generalise across taxa

The second potential issue with the use of physiological metrics as proxies for ecological performance is that the interpretation of these indicators is frequently based on the results of microalgal studies. This is presumably due to the prevalence of laboratory studies involving microalgae (which itself is probably a reflection of the logistical challenges associated with culturing large quantities of macroalgae). However, physiological interpretations derived from one taxon cannot necessarily be generalised broadly across others. This point is particularly well illustrated in examining the utility of RNA:DNA ratios as a proxy for growth rate in macroalgae. As rapidly growing organisms require more protein synthesis machinery (in the form of RNA-rich ribosomes) per unit biomass, RNA concentrations should be positively correlated with growth (Gorokhova et al. 2007). When normalised to DNA concentration, RNA:DNA ratios have been shown to closely mirror somatic growth changes in a variety of different organisms from bacteria (Kemp et al. 1993) to fish larvae (Catalán et al. 2006), zooplankton (Yebra et al. 2011) and higher plants (Reef et al. 2010b). RNA:DNA ratios have also been successfully used as a proxy for growth rate in microalgae (e.g. Schlesinger & Shuter 1981, Dortch et al. 1983, Lepp & Schmidt 1998), but the efficacy of applying this technique to macroalgae has only recently been investigated (Reef et al. 2012; Chapters 2, 3 & 4), and preliminary results of these studies suggest that RNA:DNA ratios are not directly linked to growth of macroalgae. Further studies examining the role of RNA:DNA ratios in macroalgae are clearly required, but it seems that the precedence set by microalgal research in this area may not apply to macroalgae. This illustrates the importance of exercising caution when considering ecological interpretations for seaweed based on a precedence set by microalgae (or even by other seaweed taxa in a different biogeographic context). We recommend that any novel performance metrics previously untested on seaweeds should be validated against other well-established physiological indicators wherever possible to avoid potential errors resulting from generalisations across taxonomic groups.

Issues with using proxies: multiple levels of assumptions

The final potential issue with the use of proxies discussed by this review – and perhaps the most concerning in the sense of possibly misleading ecological interpretations – is the suggestion that knowledge of one aspect of an organism's physiological response to its environment can be used to derive broad-scale ecologically meaningful interpretations. That is, the assumption that 'performance' as measured by a single aspect of physiology, e.g. nutrient uptake (Pedersen et al. 2009), chlorophyll content (Miller et al. 2006), $\delta^{13}C$ values (Cornielsen et al. 2007) or electron transport rate (Edwards & Kim 2010), can be interpreted exclusively to infer ecological success. In some cases this extrapolation may prove to be correct; for example, electron transport rate in photosystem II often closely mirrors photosynthetic rate, productivity and growth (Beer et al. 2000, Longstaff et al. 2002). Similarly, high levels of tissue % N (or rapid N uptake under non-limiting nutrient conditions) are frequently associated with rapid growth under high light conditions (Reef et al. 2012, Wang et al. 2014). However, physiological proxies provide a snapshot in time that typically describes a single physiological process, and thus they do not always accurately represent the net output of multiple simultaneous metabolic and biochemical processes (ultimately culminating in growth and productivity).

An important example of the dangers of extrapolating physiological data to infer ecological performance is in the use of maximum quantum yield (F_v/F_m) of chlorophyll fluorescence to inform on organism-scale health of seaweeds. F_v/F_m gives a measure of the proportion of open reaction centres in PSII available for photosynthesis (i.e. the intrinsic potential quantum efficiency of PSII) and is widely considered to be a sensitive measure of photosystem health (e.g. Jones et al. 2000, Ralph & Short 2002, Macedo et al. 2008). It follows that a photosynthetic organism with poorly functioning photosystems will have limited capacity for growth, which has led to some studies using F_v/F_m as a rapid measure of seaweed 'stress' (e.g. Parkhill et al. 2001, Baumann et al. 2009, Figueroa et al. 2009). While F_v/F_m undoubtedly describes the photosynthetic efficiency of PSII in its fully re-oxidised state at the instant of measurement (Ralph & Gademann 2005), recent work has demonstrated that relatively high values of F_v/F_m can be measured immediately adjacent to severely necrotic tissue (Chapter 2; see also Ralph & Short 2002 for seagrass), and F_v/F_m need not be indicative of performance at the thallus-scale.

Using F_v/F_m as an exclusive indicator of instantaneous (or even potential) ecological performance requires significant assumptions to be made about the link between the potential quantum efficiency of PSII and downstream processes, including the quantity of photosynthates generated and the direct use of photosynthates in growth. While it can be reasonably assumed that an alga cannot photosynthesise and grow without efficient absorption and transfer of light energy, high values of F_v/F_m do not necessarily indicate photosystem functionality as the transfer of light energy from reaction centres can be interrupted before PSI is even reached. For example, microalgal studies have demonstrated that heavy metals can interrupt the electron flow between the PSII plastoquinone electron receptors Q_A and Q_B (El-Sheekh et al. 2003), and the targeted use of photosynthetic electron transport inhibitors has shown that low level desiccation can significantly inhibit Calvin cycle activity without any detectable effect on F_v/F_m (Chen & Hsu 1995). Moreover, even if absorbed light energy is successfully used to generate photosynthates, these may be used in the synthesis of cellular compounds such as pigments or protective stress proteins unrelated to growth (e.g. Cruces et al. 2012). Thus, while low F_v/F_m indicates photosystem stress (short-term and rapidly reversible result of dynamic photoinhibition, or permanent and irreversible consequence of chronic damage to PSII machinery), high F_v/F_m does not necessarily translate to overall 'health' of the alga nor to growth or ecological vigour. Therefore, F_v/F_m should routinely be considered in conjunction with growth measurements if meaningful interpretations beyond the immediate state of PSII are to be made.

Summary and future directions

The first step to predicting the likely impacts of climate change on seaweed communities is determining the way in which individuals respond physiologically to their environment, and understanding how this manifests ecologically. While it is often logistically easier to use physiological proxies in place of direct ecological measurements, this review has explored some of the assumptions and levels of uncertainty involved with the use of proxies, and cautions against over-extrapolating from otherwise valid and important physiological insights. Thus, while physiological information is essential to understanding the processes ultimately determining ecological performance (as explored in Chapters 2-4 of this thesis), it should be used in combination with (not as a replacement for) direct measurements of growth and/or productivity. For questions that are ecologically focussed, we suggest that researchers should continue to measure these fundamental ecological parameters directly and only use additional physiological indicators (nucleic acids; tissue ratios of C, N and P; parameters

derived from PAM fluorometry etc.) as complementary techniques pointing to mechanisms underpinning ecological performance.

The use of broad-scale predictive tools such as BEMs is likely to increase in the future as the ecological impacts of a rapidly changing oceanographic environment become more evident. However, latitudinal variation in the physiological tolerance of seaweed to various environmental drivers (Serisawa et al. 2001, Mohring et al. 2014; Chapters 2 & 4) may preclude the utility of traditional correlative approaches, even if ecological performance can be assessed using a robust combination of growth and physiological measurements. For example, classic BEMs predict that forecast levels of climate change will likely result in southern contraction of the northern boundary of *Ecklonia radiata* in southeastern Australia, and have little effect on the ecological performance of *E. radiata* in Tasmania (near the southern extent of the species' distribution). However, Chapter 4 demonstrates that the effect of high summer temperature in combination with high levels of irradiance at high latitudes – at least in shallow waters – may result in forecast levels of ocean warming having a much greater effect at high latitudes than is widely assumed. Therefore, in order for BEMs to be a useful tool to predict future seaweed distributions under environmental change, models must incorporate additional physiological information (such as minimum metabolic requirements, the nature of interactions between multiple environmental stressors, and latitudinal clines in thermal tolerances and optima for growth and photosynthesis). A multivariate approach to measuring these aspects of seaweed physiology is essential to adequately capture complex and potentially interactive biochemical processes that may vary between species, between individuals within a species, and on different time scales within individuals.

Better integration across physiological, climatological and ecological research disciplines is clearly required to improve linkages between the seaweed physiological literature and more ecologically oriented studies. However, even if the effects of complex environmental change on the ecological performance of seaweeds can be determined precisely, their considerable ability to acclimitise to new conditions via morphological (Wernberg & Thomsen 2005, Fowler-Walker et al. 2006, Stewart 2006) and physiological (Wernberg et al. 2010; Chapter 3) plasticity may imply a low capacity for genetic adaptation. If so, it is particularly crucial to understand the organism-level responses to environmental change as this will largely dictate their success at the population level. Moreover, changes in the growth, reproductive success and survival of habitat-engineering seaweeds will likely have a secondary indirect influence

on the performance of conspecifics due to environment-engineer feedback (Cuddington et al. 2009). Although recent work has suggested that feedback between kelp thinning and post-settlement success of conspecifics is likely to be positive (Flukes et al. 2014, as Chapter 5), the mechanisms of environment-engineer feedback remain poorly understood and may be further confounded by additional changes to the understory canopy community (Flukes et al. 2014). Quantifying the extent of physiological plasticity in seaweeds relative to their capacity for genetic adaptation, and determining the effects of potential thinning of seaweed canopy on its own survival, growth and reproductive success, remain important areas of future research to fully understand the impacts of environmental change on ecosystem engineering seaweeds and associated rocky reef communities.

Appendices

Appendix A. Determining the correct duration of dark-adaptation of macroalgal tissue before initiating Rapid Light Curve sampling

While ambient light-acclimated Rapid Light Curves (RLCs) demonstrate the immediate light history of a thallus and can be affected by recent canopy shading, water clarity, cloud cover etc., dark-acclimating a thallus before initiating a RLC 'standardises' the pre-manipulation light history and reflects the inherent physiological state of photosystems beyond the immediate light history (Ralph & Gademann 2005). Thalli should be acclimated for sufficiently long to allow full re-oxidation of the electron transport chain and relaxation of photoprotective mechanisms, i.e. further dark-acclimation should result in no further increases in F_m' or $\Delta F/F_m$' (see Ralph & Gademann 2005 for explanations of terminology), but not so long that they become C-limited or so that other metabolic processes are restricted. The goal is therefore to determine the minimum duration of dark-adaptation beyond which there are no further decreases in or increases in F'_o or $\Delta F/F'_m$.

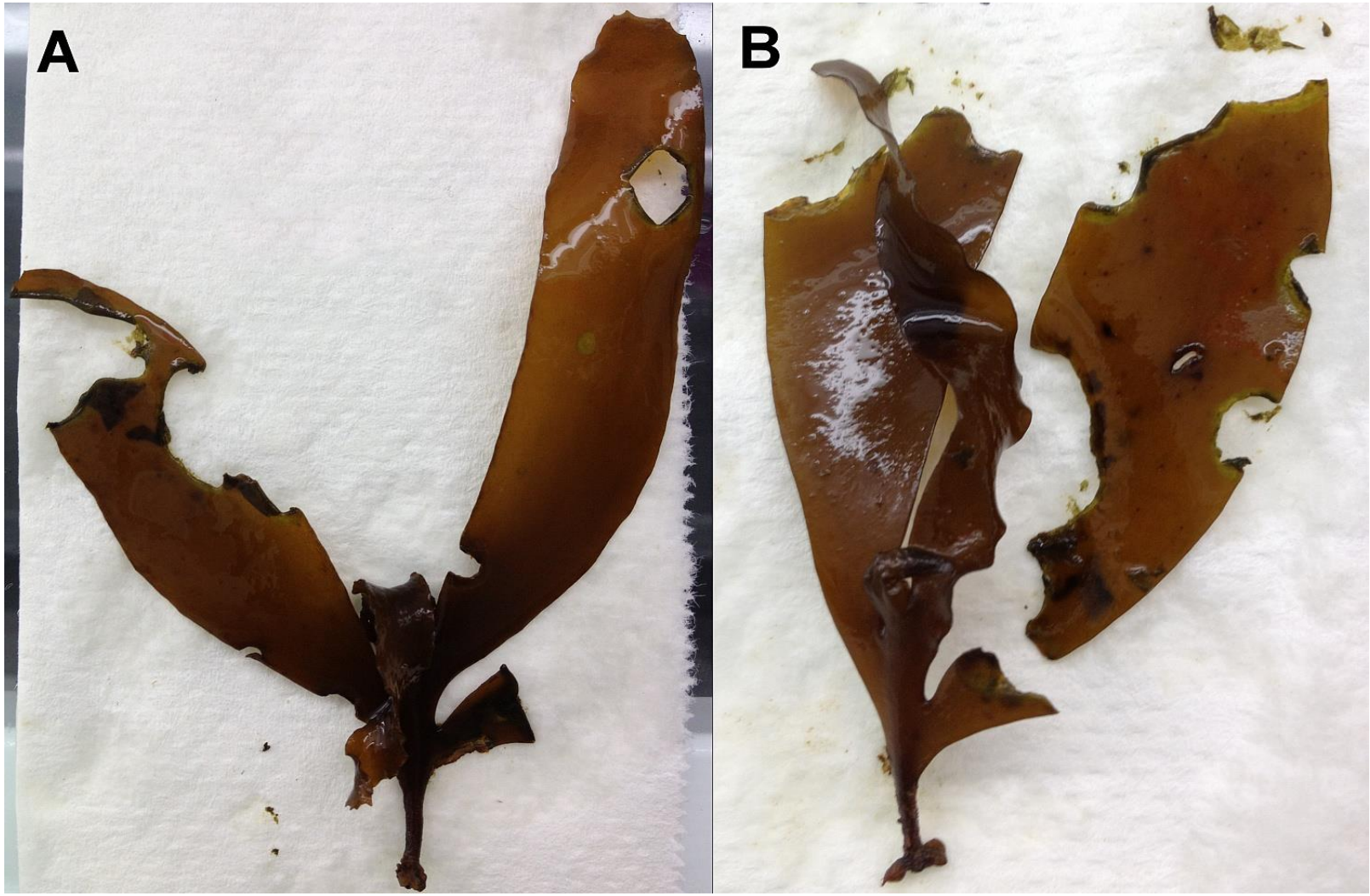
Thalli should be sampled under conditions similar to those required for final determination of light curves. If a species is to be sampled with PAM fluorometry in a laboratory experiment, thalli should be maintained at similar irradiance, temperature and flow conditions as those intended for the experiment (as flow rate can affect other processes such as rates of boundary layer diffusion; Cornwall et al. 2013).

To determine the correct duration of dark-adaptation, connect the fiber optic of the PAM fluorometer to the algal tissue, initiate a saturating pulse, and record F'_o, F'_m and $\Delta F/F'_m$. Commence dark-adaptation of the area of tissue to be sampled. This can be achieved using a "leaf clip" (termed by Walz) with the slide window closed to dark-adapt a specific area of tissue, or the entire thallus can be maintained in darkness (although the same area of tissue should be sampled each time a saturating pulse is initiated). Begin timing the duration of dark-adaptation and repeat saturating pulses (and recording of fluorescent parameters) every ~60-90 seconds. The minimum fluorescence (F'_o) should decrease with each successive saturation pulse as an increasing proportion of reaction centers open, while F'_m should remain relatively consistent. This will result in increasing values of photosynthetic yield, $\Delta F/F'_m$.

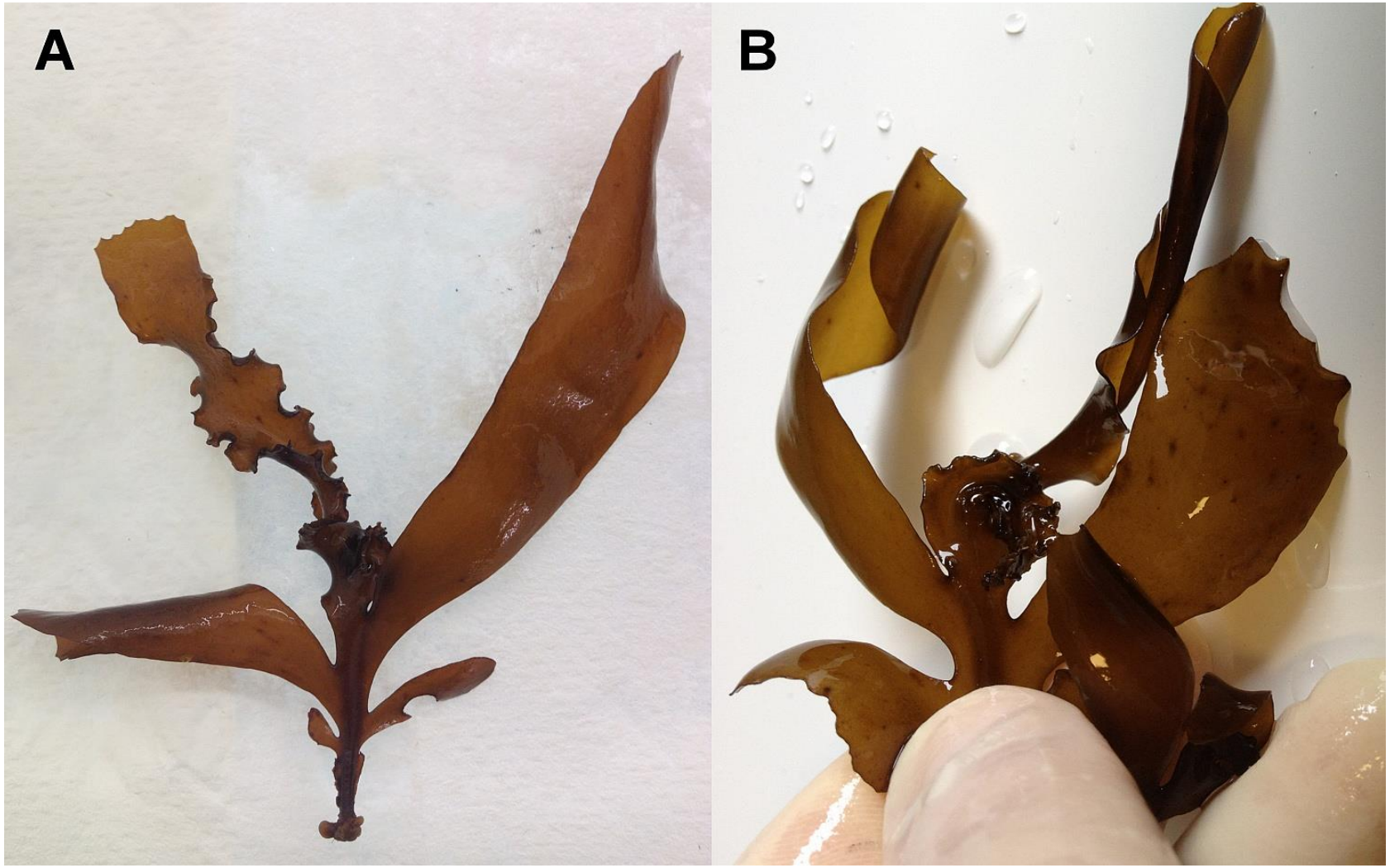
Continue sending saturating pulses every ~60-90 seconds until F'_o stops decreasing, and/or F'_m begins to decrease (which can occur if the tissue becomes O_2-limited due to attachment of the leaf clip, but typically will not be problematic for dark-adaptation durations < 30 minutes).

Once $\Delta F/F'_m$ has stabilised between successive saturation pulses, this duration of dark-adaptation represents the optimal for that thalli, but can be applied as a general 'rule of thumb' across all individuals of this species. Optimal durations of dark-adaptation are typically in the range 15-25 minutes (e.g. Wernberg et al. 2010; this thesis), although this can vary depending on algal species and past light history of a thallus. Yield measurements of tissue subject to this period of dark-adaptation represent maximum quantum yield, i.e. the maximum potential photosynthetic efficiency of photosystem II, and is denoted F_v/F_m.

Appendix B. Tissue necrosis of juvenile *Phyllospora comosa* originating from (A) NSW and (B) Tasmania following 28 days of growth at 22 °C.



Appendix C. Abnormal ‘buckled’ growth of *Phyllospora comosa* originating from (A) NSW and (B) Tasmania following 28 days of growth under laboratory conditions with minimal water movement and non-directional irradiance.



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